

T H E S I S

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on

LIFE HISTORY STUDIES of the SPECIES of
PHOMOPSIS occurring on CONIFERS

BY

GLENN GARDNER HAHN, B.Sc., M.Sc.



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LIFE HISTORY STUDIES OF THE SPECIES OF
PHOMOPSIS OCCURRING ON CONIFERS.

I N T R O D U C T I O N.

An investigation of the life history of forms of the genus Phomopsis, occurring on conifers, in all their phases has been undertaken in order to determine the identity and relationship of the various species cited in the literature. This research is an important part of a phytopathological problem which has a particular economic bearing (4, p. 7). The differentiation of Phomopsis Pseudotsugae, Wilson, the attributed cause of the Phomopsis disease of Pseudotsuga Douglasii, Carr. and other conifers (76) from similar forms of the same genus occurring on Douglas fir, became a very important mycological problem.

The purpose of the present research embodied in this paper resolved itself into the formulation of a ground work for the study of P.Pseudotsugae and all the other known species of Phomopsis on conifers, with particular reference to the Douglas fir host. On the basis of previous observations and work performed by various mycologists and pathologists, including that contributed by WILSON and

by/

by HAHN (25,76,77) it was clearly evident that the Douglas fir was a host for apparently several closely allied Phomopsis species. The original descriptions of these organisms were in given instances very inadequate and confusing; furthermore certain species were apparently so variable, that there was no sure way of identifying a Phomopsis isolated from the Douglas fir, with a previously described member of the same genus.

Whether more than one species of Phomopsis occurred on the Douglas fir, and, if more than one, on what basis could they be separated, was the question, which in the opinion of the writer, needed to be definitely settled in order to carry out fully and comprehensively pathological work with Phomopsis Pseudotsugae, strongly suspected of being a parasite. For this reason numerous forms of Phomopsis were collected widely during the period 1926-28 in Great Britain and from the continent for the purpose of cultural life-history study. This collection of forms was augmented by a collection of forms previously isolated (24) and maintained in culture, from various parts of the United States. A comparative cultural study has been made of the forms from both sides of the Atlantic under approximately the same growth conditions.

SCOPE OF THE WORK.

The research was confined to the study of all the Phomopsis species known, up to the present time, to occur on conifers, (with one possible exception, - Phomopsis Cryptomeriae, Kitajima et Kamei, which will be discussed later) and to a consideration of the perfect stages of these species. The comparative study of forms was concerned mainly with those morphological and physiological characters which seemed to be most important and practicable for a natural differentiation of the Phomopses. Special attention was given to the presence and type of each kind of spore (e.g. A, or alpha spores, B, or beta spores, and intermediate spores). The type of fructification, colour production in artificial culture, and culture growth characteristics were found to be of great importance in classifying forms. Size of spores, shape of spores, shape of extremities of spores, size of conidiophores, were also found to be of considerable importance in the classification of forms and their segregation under specific heads.

Some investigation has been done on the action of coniferous species of Phomopsis under different environmental conditions, and also with respect to their pathogenicity. In regard to the latter/

latter the following facts should be emphasized:

- (1) Healthy tissue of Douglas fir (Pseudotsuga Douglasii) artificially inoculated with Phomopsis Pseudotsugae yielded positive results, indicating the parasitic nature of this species¹.
- (2) Inoculations of healthy tissue of Douglas fir with Phomopsis occulta, Trav., and with P. conorum, (Sacc.) Died., gave negative results.
- (3) Inoculations of healthy tissue of Abies pectinata, D.C. (silver fir) with Phomopsis abietina, (Hart.) Wilson et Hahn, gave negative results.
- (4) Cankers on the main trunk or branches of healthy trees of Douglas fir which have been fully described and figured by WILSON (76), and attributed by him to Phomopsis Pseudotsugae, were invariably associated with that organism. P. Pseudotsugae was generally found fruiting abundantly on such lesions.
- (5) Three Phomopses, - Phomopsis Pseudotsugae, P. conorum (52, p. 567; 25) and P. occulta fruited commonly on the die-back lesions of small shoots both of large trees and nursery or plantation stock of Douglas fir, the plant parts of which were known to have been primarily affected by some other environmental cause which had predisposed disease.
- (6) The silver fir bark fungus, Phomopsis abietina, "Tannenrindenpilz", which is associated with a girdling disease (29) of the smaller branches of Abies pectinata, D.C. in Germany and France, was not observed in Great Britain or Scandinavia. The characteristic symptomatology of this disease/

1.

Inoculation experiments with Phomopsis Pseudotsugae have been made in a Forestry Commission plantation of Douglas fir at Glentress, Peebles-shire, Scotland, the inoculation work being performed in collaboration with Dr. Malcolm Wilson. The results of these experiments will be published jointly with Dr. Wilson at a later date.

- (6) disease on the fir host has not been recorded by pathologists in the two latter countries.
- (7) Phomopsis juniperovora, Hahn (23, 24) a serious parasite on nursery stock of species of Cupressaceae in the United States was not discovered in Great Britain.
- (8) Certain of the Phomopsis species studied appeared to be restricted to individual plant parts, and to individual hosts; other species showed avidity to infect a number of different plant parts, - trunk, lateral branches, leaves, cones, - and to occur on a wide range of hosts, e.g., P. occulta was observed to occur on species of fourteen coniferous host genera.
- (9) Coniferous Phomopsis species which manifested a wide host distribution indicated possible relationships with Phomopsis form on broad-leaved hosts.

METHODS OF PROCEDURE.

Source and Methods of Isolation of Forms.

The forms of Phomopsis presented in this paper were isolated from different conifer hosts freshly collected in nature. Usually they were obtained from the main stem, larger branches, or laterals. (1). In cases where the organism was suspected of pathogenicity, isolations were made from spores taken from pycnidia in direct association with a definite lesion, or from diseased inner tissue known, very probably, to be infected with the organism in question.

In the majority of cases isolations were made either from single spores, or from spore horn (tendrils) plantings on plates of very clear corn meal agar containing 2% cane sugar (saccharose). In the first case, an extremely dilute spore suspension in a few drops of sterile water was poured over the upper surface of the cooled and hardened corn meal medium in a Petri dish. Just enough spore suspension liquid was added to cover the agar with a thin film which was spread by gently tipping and rotating the plate to procure an even and complete distribution of the spore suspension drops. After 24 hours the dilution plates, which had been set aside in a culture cabinet/

(1).

In several instances forms were secured from cone scales.

cabinet at ordinary room temperature, were examined under the low power of the microscope. When singly occurring spores were observed to have germinated, these were indicated by a minute incision round about in the agar, made with a sterile needle. The plate was then quickly removed to a dissecting microscope and the marked germinated spore completely blocked out with a fine tip of a sterile scalpel. To make certain that only one spore was isolated by this process the isolated block or "island" was again examined under the microscope, this time under the high power to determine, absolutely that only a single spore was present. The isolated block was then removed with a finely pointed, polished needle to a tube of corn meal agar. Generally eight or a dozen single spore isolations were made of a given form. These were all compared culturally and differences watched for, to detect if possible any particular aberrances in colony growth characteristics or colour production. It was the general experience throughout the culture study, that such single spore isolations showed excellent agreement with each other. The writer found that single spores could be isolated most expeditiously by the above method, in a ordinary laboratory room where air/

air draughts had been eliminated. Contamination of the isolated spore transfers was not experienced, despite the fact that the plate, while being observed microscopically had the cover removed.

Where cultures were obtained from spore horns the following process was followed. The pieces or bits of host material exhibiting the fructifications of the Phomopsis were placed on moistened filter paper in a closed dish, which had previously been sterilised either by heat, or cleansing with a 0.1 per cent corrosive sublimate solution followed by sterile water. When the spore horns were extruded under these moist conditions, a process which usually happened very quickly, providing fungus material in a mature condition was being dealt with, they were lifted on the point of a sterile needle which just previously had been placed below the agar surface of a Petri dish, prepared for the growth of the spore tendrils. The bit of agar adhering to the needle caused the spore horn to stick to the instrument, particularly if the tendril had become dry and brittle. The individual spore tendrils were distributed in the Petri dish. When these had formed colonies, sub-cultures to individual tubes were made by transfers of bits of the advancing edge of each colony./

colony.

In certain instances isolations were made by means of single colony dilution plates and from affected tissues of the host. In the latter instance the affected part of the host was first thoroughly wiped with a piece of cotton wool moistened in 0.1 per cent. solution of corrosive sublimate, and then the epidermis and part of the upper cortical tissues cut away with a sterile scalpel. A small fragment of diseased tissue was then cut out, and removed immediately by means of a sterile needle to cooled poured plates of a suitable medium. The following process has also been used. The affected host part was dipped in 95% methyl spirits, touched to a flame and allowed to burn for an instant; then the upper tissues were cut away under aseptic conditions, and cultures made from bits of the exposed tissues. In certain instances where an extremely small diameter was being dealt with, (e.g. the main stem of nursery seedlings or transplants) the stem was quickly and briefly ignited, after dipping in alcohol, and then the flamed tissue containing the fungus was planted directly, partly below the surface of the agar medium. In the last process it was found that despite the drastic measures of outer sterilisation, the fungus generally sustained the ordeal, and grew out eventually from the inner tissues.

After/

After several days the growth characteristics of sub-cultures were compared, both with each other, and with other culture forms which they were considered to resemble in nature. The first series of sub-cultures, obtained by transfers from original isolations, was generally pure.

Culture Media.

Culture growth studies of Phomopsis juniperovora (24) indicated that this species and closely related forms (25) grew and fruited readily on sugar - corn meal agar. WEHMEYER (67, p.245) and ARCHER (1) had also observed as a result of their extensive culture studies of species amongst the Sphaeropsidales, that hard oatmeal agar and Leonian's agar gave splendid results, both being media upon which one could be certain of obtaining pycnidia. Upon the basis of previous work performed by the writer and upon that done by Wehmeyer and Archer, it was decided to confine the present cultural study of coniferous Phomopsis forms to the three media cited above. They will accordingly be referred to as sugar - corn meal agar,¹ Leonian's agar,² oatmeal agar.³

1.

Essentially that given by Harshberger (Text book Myc. and Plant Path., 1918, p.608); 2% agar was used. After the final filtering 2% cane sugar was added and stirred until completely dissolved; autoclaved for 15 minutes at 15 pounds pressure. This medium usually titrated +3.5, but a little variation did not seem to affect growth.

2.

That given by Leonian (Am.Jour. Bot. Vol.XI, 1924, p.20-21) as follows; a nutrient solution plus 2% agar - KH_2PO_4 , 1.25 gms; MgSO_4 , 0.625 gm; peptone, 0.625gm.; maltose, 6.25 gms; Malt extract, 6.25 gms; distilled water, 1,000 c.c.

3.

That given by Pethybridge and Murphy, (Sci.Proc.Dub. Soc. Vol. XIII, 1913, p.580), as follows: ground Quaker oats, 60 gms; cold water, 1,000 c.c.; agar, 2%.

It is a well known fact among mycologists and plant pathologists that many fungi vary considerably in their macro and microscopic characters under different environmental conditions. The work of COONS (10) and LEONIAN (37) have shown that the Sphaeropsidales do vary greatly in response to the environment. It is now recognised that a great deal of the disagreement in the past between workers, when one man has attempted to repeat the results of another, has been due to these morphological variations under different sets of conditions. As ARCHER (1) has pointed out, the very nature of these variations, indicates clearly the need of a standard physiological basis by which we may make morphological comparisons between various types of fruiting structures. Upon such a basis, alone, can a rational taxonomic classification scheme be formulated whereby fungus species may be recognised by characters appearing under certain definite prescribed conditions.

The development of a nutrient medium by LEONIAN (37) tended to fulfill a long felt need. In this medium a hard agar is obtained with a high food concentration of organic and inorganic substances, favourable for both pycnidial and perithecial development. However, so far as the present coniferous Phomopsis culture study was concerned, the forms were observed to fruit in certain instances more readily upon the sugar-cornmeal and the oat agar media than upon/

upon Leonian's agar.

The best perithecial development was obtained on "natural media", i.e., twigs of various conifers and broad-leaved hosts, - Ulmus campestris, L., Alnus Sitchensis, Sarg., and Acer Pseudoplatanus, L. All twigs for culture purposes were placed in test tubes containing 5 c.c. of water, and sterilised at 15 pounds pressure for 2 hours. This use of a more solid substratum for perfect stage production, was also conducive to the imperfect stage production.

Treatment of Pure Cultures.

During the present research it was found that the Phomopsis forms generally could be maintained in a condition of normal growth by continued subculturing on hard oat agar. A normal culture was considered one in which the A type conidia (fusoid spores) were abundant and typical in size and shape; also one in which the culture growth characteristics appeared, which were peculiar to, and typical for, the fungus when freshly isolated on that particular medium. To ensure vigorous cultures of certain forms it was necessary to subculture frequently (every second or third month) on oat agar; other forms did not appear to suffer by neglect due to delayed subculturing. Non-vigorous cultures could sometimes be revived by transfers to oat agar. Certain forms, however, failed to respond to such a treatment. The best policy seemed/

seemed to be to maintain stock cultures of forms being studied, on oat agar. When culture growth was desired on other media, transfers could then be made from these stock cultures.

It was found that certain forms if grown continuously on rich nutrient agar, or on media in which there was an excess of water, appeared to undergo a form of degeneration, and a shortened duration of the vitality of the cultures resulted. Such cultures which had "run out", lost the characteristic growth characteristics, producing colour reactions quite unlike those obtained when the organism was freshly isolated. Furthermore, the ability to produce fruit bodies in such degenerate cultures seemed to disappear entirely, the fungus culture becoming sterile. It was frequently observed that in cultures which had departed from the "norm", - a descriptive term used by WOLLENWEBER, SHERBAKOFF and others (81) for the type of culture development, which produces to an optimum degree all the phases of fungus^{growth} - the mycelium produced a gelatinous type of growth; in certain cases an immersed type of growth of the mycelium predominated, accompanied by sterility of the culture. Fortunately the number of conifer Phomopsis showing a tendency to degenerate was comparatively small. 0/

Cultures were kept at ordinary room temperature, and care was taken not to expose them to direct sunlight/

sunlight, since under such conditions the culture tubes sweated badly, rendering the reading of growth characteristics most difficult.

The twig cultures ("natural media") inoculated for the purpose of the production of the perfect stage were placed in cold storage when fungus growth had just started and kept at a temperature maintained practically at the freezing point, (fluctuating slightly at certain periods to 29° F., and not above 34° F.). These twigs were left in storage for three months and then removed to the laboratory when they were placed under moistened conditions. The tubes were later removed to a cold greenhouse where they were protected from direct sunlight.¹.

Labelling Cultures.

Isolated cultures of Phomopsis forms bore the collection number of the specimen from which the isolation had been made. The collection card accompanying the specimen, therefore, contained the history of the fungus from which the isolation strains had been made.

Usually/

1.

For the sake of coherence, other methods of procedure involved in the treatment of cultures, particularly with regard to experimentation in developing the perfect stage, will be given under the discussion of Diaporthe conorum and certain Phomopsis species.

Usually six or more isolations were made of a single form. Each individual isolation bore the collection number of the specimen, followed by the first letter of the alphabet (capitalised); sub-numbers indicated the individual forms isolated, e.g. 43943 A¹, implied the first culture generation, form No. 1, isolated from specimen 43934. The second culture generation of form No. 1 was written so, 43943 B¹. In this way a record could be kept of the culture generation history of the culture. Supplementary data with regard to date of the transfer, medium used, and the culture growth characteristics, were kept on individual cards for each culture form. When the perfect stage (Diaporthe) was isolated in pure culture, Roman numerals were substituted for the Arabic sub-numbers.

METHOD OF STUDY AND PRESENTATION.

Throughout the study of the morphological and physiological characteristics of the conifer Phomopses, wherein their constancy or variability was investigated, pedigreed strains of the various organisms were cultivated on both natural and artificial media. In a given comparative culture test of a group of forms, the strains were transferred to the medium upon which their reactions were to be studied on the same day, and the whole set of cultures were kept under as nearly the same environmental conditions as was possible. Duplicate, triplicate, and even more tests were made to make sure that the results obtained in the first instance were typical and constant. The inocula used in the experiment ^{etc} was selected with care. Where possible, vigorous bits of submerged, as well as aerial mycelium, involving a certain number of fruiting bodies, in cases where such were obtainable, were used in making sub-cultures. It was frequently found that where the inocula contained spores, the sub-cultures showed a greater tendency to produce spores in turn, than sub-cultures which were obtained from non spore-bearing mycelium.

In the macroscopical examination of the culture, special attention was given to the amount and the character of growth, of the mycelium in the aerial stratum; to the colour of the aerial and submerged/

submerged hyphal stratas; to the occurrence of crystals in or on the medium, formed as a result of the growth of the particular fungus form on the medium; and to the types of stromatic fructifications appearing just beneath, or on the surface of the middle stratum.

In the microscopical study, the different types of conidia (A, B, and intermediate spores), and conidiophores, received special attention. The observations were recorded by means of camera lucida drawings, and by necessary measurements.

All drawings of spores were made of either dead, or living freshly exuded material mounted in a glycerine mounting medium containing potassium acetate,¹ under the oil immersion lens, and with the xl2 ocular. In making the camera lucida drawings it was the author's desire to depict the apical and basal ends of the conidia as accurately as possible; every type of conidium occurring was illustrated but the typical and exceptional cases were designated separately. The microscopical character of the mycelium, so far as observed by the writer, could not be used in the specific differentiation of forms; therefore little attention was given to it.

1.

Mounting fluid plus erythrosin to colour: 1 part 2 per cent. potassium acetate solution; 1 part 40 per cent. glycerine in ethyl alcohol; copper acetate to colour just slightly; add erythrosin until a rich colour is obtained.

Photographs of fruiting bodies were made of material sectioned on the freezing microtome, stained with cotton blue and mounted in glycerine jelly.

Pycnidiospore measurements were made whenever possible of mature spores, which had been exuded from the pycnidium in a spore tendril or droplet. Ascospores were obtained from the perithecia in fresh material by dissecting away the surrounding cortical tissues of the host and exposing the flask-shaped bodies, which were then easily burst by slight pressure with a sterile needle, whereupon the ascospores were ejected in a mucilaginous globule. Spores from exsiccati could only be obtained by crushing fragments of the material.

Measurements were made with the high power objective and an eye-piece micrometer. Care was taken not to measure spores in the same field twice. At the outset, before commencing to measure, a number of fields were examined in order to estimate the prevailing size and shape of each spore type to be measured. When the particular type showed little variability in shape and size, only ten conidia of each were measured; when the material showed considerable variability, at least twenty spores or more of each important type were measured. The averages and extreme ranges of these measurements were recorded, both for the particular fungus form, and for the entire series of forms studied.

In/

In differentiating forms where biometric comparisons were utilised, not less than one hundred measurements of a given spore type were made. Biometrical comparisons were recorded according to the method quoted by ROSENBAUM (51, p.268) from Reitz and Smith. Such comparisons were based on the relation of the difference of the means, to the probable error of the difference between these means, and indicated any significant difference which might exist between spore populations of the forms being studied.

Where a new species was being described, at least one hundred measurements were made. These measurements as presented for the particular species, included both the extreme range, and the common range, - the size range in which most of the spores occurred. Both these ranges are important; for it was found that the spores of conifer Phomopses do vary, but within a specific range which was more or less constant.

THE GENUS PHOMOPSIS.

The genus Phomopsis has received considerable attention from mycologists and pathologists partly on account of the comparatively large number of pathogenes which the genus includes and partly due to the fact that many of its species produce the two types of spores A and B. The genus appears to be fairly well founded, particularly when one considers alone the cultural connections obtained by WEHMEYER (67,68,69, 70, 71, 72, 73) who has demonstrated repeatedly that the imperfect stages of the species of the Diaporthe group are strikingly constant, being almost universally, species of the genus Phomopsis.

The description of the genus Phomopsis as originally considered may be stated in brief as follows:- Sphaeropsidales, Sphaerioidaceae, - Hyalosporae with stromatic pycnidia with a single chamber in which occur "basidiis uncinatis"; the spermagonial stage of Diaporthe.

Historical Review of the Genus Phomopsis.

As originally erected, the genus Phomopsis was merely a division of the very large genus Phoma, designated in 1884 by Saccardo (56,III, p.66) to contain those species with "perithecia subastomo depresso, basidiis demum uncinatis instructae (Phomopsis) spermogonia Diaporthe sistentes".

In/

In 1905 this characteristic section was definitely split off by the same author (57) as a new genus with the following description:-

"Pycnidia subcutanea, plus minus erumpentia, globoso-depressa, saepe longitudinaliter oblonga, non raro supra latiuscule aperta nec regulariter ostiolata, nigricantia, gregaria. Sporulae fusoides-oblongae rarius ellipsoideae, typice 2-guttulatae. Basidia filiformia v. acicularia, saepe demum secudentia et incurvata. - Huc spectant numerosae Phomae species veluti pycnidia Diaporthes habitae." At this time as GROVE (20) pointed out the real basis of the sub-division was imperfectly understood.

In 1903, von HÖHNEL (30) had erected a genus amongst the Melanconiaceae, - Myxolibertella: "Est Libertella vel Myxosporium cum sporulis filiformibus et oblongis (vel fusoides) commixtis". Two years later BUBÁK (7) stated that he regarded Phomopsis Lactucae (Sacc.) Bub. as having both fusoid spores and "Septoria-artigen sporen" which were bent at the extremity "hakenförmig" and borne on short somewhat stout cone-shaped sporophores. SACCARDO (57) commented on Bubák's conception of the filiform spores occurring in the genus Phomopsis, but he himself is not clear/

clear concerning the origin and function of these "basidia fuliformia," as he would call them.

von HÖHNEL (31) a year later (1906) placed his new genus Myxolibertella as a synonym of Phomopsis. He was strongly opposed to Saccardo's view of the filiform structures of Phomopsis as being regarded as separated basidia. He regarded them instead as very definite "stylospores", and pointed out that these had been previously rightly designated by the Tulasnes, Nitschke and Fuckel as such. With regard to the fruit body of Phomopsis von Höhnel regarded it only as a cavity in a young stroma which arose before the development of the perithecia, and possessed no walls of its own. He suggested that this type of fruit structure could only belong to the Melanconiales. He did not, however, continue within this opinion, for in his key of the Fungi imperfecti he did not place Phomopsis among the Melanconiales (34, p. 330).

The same year TRAVERSO (66) in Italy recognised all such forms having the fusoid and the filamentous shaped spores, as Phomopsis, and described these as the imperfect stage of Diaporthe. Traverso considered the filamentous bodies/^{as} released sporophores.

In 1911, the uncertainty surrounding the genus was completely cleared up by DIEDICKE (13) who brought out the well-marked morphological characters of the genus. With regard to the "basidia filiformia" he/

he considered them to be truly spores, which he designated as "B spores", arising from definite, characteristically shaped sporophores. In shape Diedicke observed them to be quite different from the sporophores of the "A spores", occurring in certain instances alone, and not intermixed with the A spores. For the two closely associated spore types, so pronouncedly distinct in shape, Diedicke had two considerations: first, that the two types of spores were different developmental states of the same fungus, the imperfect stage of Diaporthe appearing at different times (this consideration he regarded as the apparent state of affairs), or second, that there were two different fungi concerned, (Phoma and Phlyctaena?), perhaps both belonging in the same development cycle of the same Diaporthe. These two fungi matured at the same time, and frequently were so closely associated that their fructifications fused, and their spores were thus produced in "mixed fruit bodies". Diedicke advocated with regard to this second consideration, the performance of culture studies with spores of Diaporthe, as well from single A and B type spores; for only by such studies did he consider that complete understanding of the two spore types could be obtained.

DIEDICKE (13) was quite clear with regard to the morphology of the fruit body of Phomopsis. He recognised, however, a certain range of variability in structure/

structure amongst the species of the genus. He gave the following characteristics as diagnostic for the fruit body of the genus Phomopsis:-

- (1) Fruit body stromatic, enclosed, erumpent.
- (2) Outer wall of thick-walled, blackish, or dark brown pseudoparenchymatous cells.
- (3) Inner layer of light brown radially lengthened, almost fibre-like cells.
- (4) Separated indistinctly from (3) occurred the hyaline cells of the hymenium, from which the filamentous sporophores arose.
- (5) Fruit body unilocular, or incompletely divided by the arching of the base and pushing in of the sides so that the body had a chambered appearance.

In 1915, DIEDICKE (14) wavered somewhat from his original conception of the A and B types of spores of the genus Phomopsis. At this later period he seemed inclined to speak of them merely as "forms", and in the case of Phomopsis Arctii, (Lasch.) Trav. where he found both types of spores present together, he regarded this species as abnormal.

GROVE (20) in 1917 in his monographic treatment of the British species of Phomopsis showed no hesitancy in calling the "basidiis uncinatis" of Saccardo by the terms of A and B spores as previously designated by Diedicke. Grove gave his views on what he considered to be the real difference existing between the new genus Phomopsis and its parent, the/

the old genus Phoma from which it had been cut off. Two features were regarded by GROVE (20, p. 52) as distinctive for the genus Phomopsis, (1) the permanent sporophores, and (2) the nature of the pycnidium, which he regarded as bearing little resemblance to that of a typical Phoma.

Along with Grove, other investigators generally have considered these much debated abstricted spore forms, as true spores, but a few have been inclined to use the term "free paraphyses" as can be noted in the papers by REDDICK (48), by FAWCETT (18) and by CAYLEY (9). The so called B spore which even today is so little understood, and its function in the life-history of the organism, still quite an enigma, will be further discussed as a part of the life-history studies of the conifer Phomopses.

When GROVE (20) published his monograph in 1917, he stated that proofs by culture seemed to be altogether non-existent that the form genus Phomopsis was the pycnidial stage of Diaporthe. By 1923-5, WEHMEYER (67; 68; 69; p. 577, p. 615) was able to cite a considerable number of instances where cultural connections had been obtained between the ascomycete Diaporthe and the imperfect stage Phomopsis.

The further extensive researches of this worker together with those of HARTER and FIELD (27, 28)/

(28), CAYLEY (9), WOLF (80) and the writer in the present paper, have collectively contributed further evidence that Diaporthe is to be generally considered the perfect stage of Phomopsis.

Present Conception of the Genus Phomopsis.

Thus it has come about by cultural life-history studies of species of the genus Phomopsis and closely allied genera that we have been able to obtain a truer morphological picture of that genus. In 1925, WEHMEYER (69, p.615) who has specialised more than any other investigator on both the morphology and physiology of the Diaporthaceae and their imperfect stages, wrote concerning Phomopsis:-

"The imperfect fruit body consists of a spherical, flattened, or somewhat irregular cavity, which is formed within a more or less well developed ectostroma. This ectostroma may occur isolated, or in connection with a perithecial entostroma beneath. These are two types of spores formed, either in separate pycnidia, or usually in the same pycnidium. One type of conidium is long-cylindrical, hyaline, one-celled, and usually curved or hamate; the other type is one-celled, hyaline, elliptical to fusoid, and contains two oil droplets."

In the description of the pycnidial stage of Diaporthe oncostoma, (Duby.) Fck., WEHMEYER (67 p. 248) further amplified his idea of the stromatic pycnidial body. "In the simplest and smallest stromata the cavities are irregularly lens-shaped. In the more extensive stromatic formations often formed as the smaller ones, the cavities are larger and frequently form/

form projections from the base or sides of their walls, making an irregular cavity buried in a well developed stroma. The stromata, furthermore, often become compounded and form large tuberculate masses 2-5 mm. in diameter." In the case of the life-history study of Diaporthe sp. (71, p. 388) he reported the pycnidial locules as being flattened, lenticular, spherical, or irregular in shape.

As a result of a wide study of the cultural life-histories of Diaporthe, WEHMEYER concluded (72, p. 168) "that the size, shape and structure of the pycnidial stroma, pycnidial locule and so called pycnidial wall are by no means constant, and are very unreliable as diagnostic characters, at least in Phomopsis and related form genera."

Other contributions to our knowledge of the life-history of Phomopsis has been given by ARCHER (1) who made a critical study of the stroma and cavity formation in Phomopsis Arctii and in P. coneglanensis, Trav. Archer pointed out that two factors (1) food materials and (2) moisture were conducive to the production of not only more stromatic primordia per unit area, but also to larger primordia. Where a large, solid mass of stromatic tissue was formed, this was produced by the fusion of many contiguous primordia which had a common enveloping brown layer. In this stromatic mass cavities arose at various points and if moisture conditions remained/

remained favourable these enlarged to become finally confluent, and merged into a single, large irregular cavity. On the other hand if the substratum suddenly dried out, this development ceased, the cavities remaining separate, and a multilocular fruit body resulted.

This last consideration immediately confronts the mycologist with a Fusicoccum-like fruit body and he is aware of the artificiality of the taxonomic boundaries of genera within the Sphaeropsidaecae. He realises moreover, how minute structural differences have been emphasised arbitrarily, without a proper appreciation of the variation of such structures within a given genus. One can hardly agree with GROVE (20), who in separating the genera Phomopsis and Fusicoccum, decided that when the protrusions from the stromatic walls of the fruit body became more decided the genus Phomopsis became Fusicoccum. The latter he regarded as having typically larger spores. As the present paper will show this is very probably not the case. Just where the genus Phomopsis leaves off and the genus Fusicoccum begins, can only be determined at best arbitrarily but on the basis of correlated characters in a large number of species belonging to the groups in which the genus lies. This all means a knowledge of the relationships and variations within the group being studied.

Personally/

Personally the writer knows very few Fusicoccums and therefore he would not attempt to limit the genus. According to his interpretation Fusicoccum is the imperfect stage of such genera as Cryptospora and Cryptosporella (59; 13, p. 12; 60) possessing a conidial stroma entirely ectostromatic, multilocular, tending to have in nature the cavities near the surface, or in the lateral portions of the stroma. Although certain of the species produce both A and B type spores, the latter does not appear to be so generally present as in the genus Phomopsis.

Among the large number of Phomopsis forms studied in connection with Diaporthe life-histories by WEHMEYER (72, p. 173; 73, p.226) that investigator mentioned only two species of Phomopsis producing the A type spore without the B. JENKINS(36) cited only the A type of spore as being formed by the imperfect stage of Diaporthe umbrina, Jenkins. WILSON (75) described Phomopsis Pseudotsugae with both A and B spores, but later (76) decided that only the A type occurred. While generally speaking Phomopsis has been considered a genus in which both A and B type spores are produced, this does not appear to be necessarily always the case.

CONIFEROUS PHOMOPSES - THEIR ECOLOGY.

Effect of Artificial Culture on Stroma Characters.

As has already been intimated, in the discussion of the genus Phomopsis, comparatively little systematic investigation has been done on the ecology of the Sphaeropsidales. The data on this subject which exists at the present time applies only to a limited number of species. So far as the group Phomopsis is concerned, it seems to be the general consensus of opinion, that the stromata of species of ~~the~~ Phomopsis vary considerably, in size and shape, under conditions of artificial culture. One has only to peruse the pathological literature (much of which is cited in this paper) dealing with Phomopsis parasites of various agricultural and ornamental host plants, and one becomes aware of the comparatively large number of investigators who have described and figured many sorts of stromatic structures, which they have obtained under different sets of environmental conditions. Polymorphic columnar stromatic masses, figured and described by CAYLEY (9, p. 263), and by WEHMEYER (70, p. 245) are now well known. These elongated-cylindrical, irregularly shaped plectenchymatic bodies were described by the latter worker for Diaporthe

Diaporthe obscura, (Pk.) Sacc. as bodies in which conidial locules developed at various points. The walls of these locules were composed of thick walled pseudo-parenchymatous tissue which was dark brown in colour. On the other hand pycnidia formed in culture may be thin walled and membranous, such as those described by JENKINS (36, p. 597) for Diaporthe umbrina or partially or totally embedded pycnidia, in which the wall, when the spore cavity reaches maturity, may be entirely dissolved away leaving only an opening into the agar, as in the case of artificially produced pycnidia of P. Arctii, (Lasch) Trav., described by ARCHER (1, p. 19).

The conifer Phomopsis species grown on the artificial substrata employed in this research, have shown great variability in stromatic structure. All of the forms produced compound, tuberculate, multi-locular structures varying in size particularly on oat and sugar-cornmeal agars containing plenty of moisture. Polymorphism in certain instances on hard agars and on natural media, was most striking. On the harder substrata, provided by natural twig media, the pycnidial bodies tended to form more as they did in nature. At the same time on natural media polymorphic bodies also formed. Phomopsis occult produced on elm stems elongate, slender, flexuous structures (some 5 mm. long), which so closely resembled the perithecial beaks/

beaks of the perfect stage of this fungus (Diaporthe conorum) that at first they were mistaken as such. In this slender stalk of twisted plechtenchymatic tissue, conidial locules were discovered. Abundant moisture in the medium itself, and throughout certain parts of the tube seemed to favour the production of these variously shaped structures (Plate). In tubes which appeared not to be too wet, or in the drier parts of moister tubes, flask-shaped semi-erumpent or superficial pycnidia formed, frequently provided with ostioles. In such cultures vegetative growth was appreciably lessened. With regard to the formation of so called normal pycnidia, much would appear to depend upon the proper balance of moisture within and without the substratum.

Effect of artificial culture on spore shape and size.

The artificial substratum had little or no effect in altering the shape of the spores produced in culture or their specific size. Variation in size which did occur amongst spores in culture, was always found to be within the specific range determined for the fungus. The similarity between Phomopsis spores produced in nature and in culture has been already stated by HARTER and FIELD (27) for the imperfect stage of Diaporthe batatatis, Harter et Field and by WINSTON, BOWMAN, and BACH (79) for Phomopsis citri, Fawcett. CAYLEY/

CAYLEY (9) on the other hand stated that A spores procured from mono-ascospore cultures of D. pernicios on hard agars were rather undersised (shorter and wider) than typical pycnospores to be found in nature. She did not state if this reduced size was still within the spore range of the fungus.

Effect of Light upon Culture Growth Characteristics.

With regard to culture growth characteristics it was found that the pronounced zonations in the aerial hyphae growth of Phomopsis conorum occurred both in the dark and in the light.

Cultures of Phomopsis juniperovora produced the striking empire yellow colour in diffuse light as readily as did cultures exposed to the full light. Diffuse light appeared to have little or no effect on colour production. Cultures of P. juniperovora had been kept for an extended period of five years more or less continuously in subdued light, and they still continued to reproduce the characteristic colour together with the flaming orange crystals in the medium.

CONIFEROUS PHOMOPSES - VARIABILITY.

The reaction of the conifer Phomopses to their cultural environment has just been discussed. When one compares the extreme variability of the stroma, and the constancy of size and shape of the spores borne in these fruiting structures, one is impressed with the specific nature of the reproductive bodies. It is for this reason that spore size and shape become extremely valuable as a specific character.

The size of spores produced in culture compared most favourably with those to be found in nature. Variation occurred but within the specific range for naturally produced spores. This range could be readily determined by the measurement of a sufficient number of spores from several forms on various hosts for the species.

In forms producing both A and B type spores there was a certain amount of variability in the relative production of these different types. Some forms produced both types readily, others were found to produce only one type, or the other, under the same set of environmental conditions, e.g., a form of Phomopsis occulta (№43965) isolated from the inner bark of a dead terminal of Abies pectinata, (collected in the Jura, France), produced only B spores in culture which were typical for that species.

On the other hand, forms which were observed to/

to produce only A spores in nature, continued to do likewise in culture, showing a decided constancy in this respect, e.g., Phomopsis Pseudotsugae has been observed in culture since 1923, and the species has never been found to produce the B type of spore. In all, thirty-three forms of this fungus have been studied, isolated from Douglas fir, Larix europaea, D.C., L. Sibirica, Ledeb., Cedrus Atlantica, Manetti and Sequoia gigantea, D.C. It would appear that in certain species of Phomopsis where the B spore, consistently, does not occur, that this type is not to be considered as repressed, but rather, that it is no longer existant in the life-history of the particular Phomopsis species.

The shape of the A and B spores of the conifer Phomopsis, and also the ascospores of the Diaporthe which is the proven perfect stage of one of the species, agreed generally with the shape of spores produced in culture.

The conidiophores varied from very short and abbreviated processes to longer subulate, flexuous structures, but they also appeared to have a fairly definite range. Fundamentally the general shape was the same throughout. In the case of the Phomopsis stage of Diaporthe tessella (Pers.) Rehm it is here interesting to note that WEHMEYER (73 p. 226) observed this species to produce its A spores in three different ways, (1) abstriction from the end of conidiophores, (2) "budding" directly from the walls of the hymenial/

hymenial cells without the intervening long conidio-phores, (3) endogenously, conidia forming within the disintegrating cells of the inner stromatic tissue.

Culture growth characteristics were particularly constant for the forms studied, providing the cultures were maintained in a normal condition. This matter of constancy applied, to the extent of development and type of the aerial mycelium, to the zonation of aerial hyphae, and to colour production. In regard to colour production it was observed that Phomopsis Pseudotsugae cultured for 5 years, and P. juniperovora for a longer period, did not lose their ability to reproduce the colours, characteristic of them. The latter fungus produced the flaming orange crystals repeatedly with each sub-culturing during this period of time. Crystals were observed to form on the surface of sterile twigs in culture.

The colour of the extruded tendrils from the fruit bodies showed great variation in colour varying from whitish to yellowish, becoming in certain instances, pinkish, or pale orange. The general tendency, however, was for the spore horns to become whitish or yellowish.

MORPHOLOGICAL CHARACTERS AND THEIR TAXONOMIC USE.

In classifying the many forms of Phomopsis isolated from conifers, particular attention was paid to those morphological characters which indicated the greatest prominence and stability.

At the outset of developing a scheme of classification the forms appeared to fall into two natural groups:-

- (1) Those producing both A and B type spores;
- (2) Those producing only the A type.

For a time this grouping seemed to hold until the B type spores were discovered for Phomopsis Montanensis, Hahn, n. sp., of group (2), occurring in scanty numbers.

Another character appearing in nature which seemed to separate the forms into two groups, was the structure of the inner pycnidium. This difference in structure, of the inner pycnidium was very obvious in cases where the ectostromatic fruit bodies of Phomopsis occulta, group (1), and P. Pseudotsugae, group (2), occurred on the same substratum, (shoots of Pseudotsuga Douglasii), amongst the cells of the upper cortex. In the case of all the A and B spore-producing forms, (with the exception of P. Montanensis), a single chamber or loculus usually formed in one plane; within the simple pycnidium of the non-B spore-forming group (and in P. Montanensis) very small/

small, simple primorida produced likewise only one cavity, whereas in larger primordia, several/^{small}cavities formed which tended to fuse into an irregular, unilocular chamber. In many instances this fusion was not complete, and a multilocular fruit body remained.

In other words, the non-B spore-forming group in nature appeared to resemble both a Phomopsis and a Fusicoccum-type of fungus discussed on p. 29 . With regard to these two genera, the forms of group (2), in which the simple primordium became multilocular, appeared to resemble more closely a Phomopsis-type of fungus, on account of the embedded nature of the pycnidium amongst the cells of the outer cortex. Through these tissues, and the cells of the epidermis, the fruit body became erumpent. That part of the pycnidium which was exposed became darker coloured, carbonaceous, the fungus tissue here being of varying thickness, and composed of pseudoparenchymatous hyphal cells, which were coloured dark brown (fuliginous). The pycnidial walls at the sides, and on the floor of the fruit body, were comparatively much thinner, and the cells composing them dilutely coloured towards the inside of the walls. The spores were produced on sporophores arising from the hymenium lining the cavities. From the basal part of the fruit body and sides, where the pycnidial walls were very thin, the sporophores looked as if they were originating from the cells of the host itself. This appearance/

appearance of the structure of the fruit body was quite in keeping with the generally conceived idea for a so called typical Phomopsis. Furthermore, isolated cortical cells of the host were incorporated amongst the hyphal tissue of the compound fruit body. According to ARCHER (1) this occurrence frequently happened with the compound fruit bodies of the Phomopses producing A and B spores which he investigated.

If one examines the figures of fruiting bodies given by DIEDICKE (13) in his paper dealing with the genus Phomopsis one observes how, generally speaking, these bodies are represented with a single chamber which is variable in shape. In certain instances the chambers are exceedingly irregular with protrusions from the side walls which may be regarded as partial septa. The same general type of fruiting bodies depicted by Diedicke, have been figured by HARTER and FIELD (27), ENLOWS (17), HAHN(23), CAYLEY (9), WEHMEYER (68, 71, 72, 73) and others.

On the other hand JENKINS (36) has described the imperfect (Phomopsis) stage of Diaporthe umbrina as being simple (unilocular), or chambered as the result of protrusions developing from the wall. SYDOW (65) in the description of his new species Phomopsis strobi described the fruit body as being separated up into numerous fully formed or incomplete chambers of various shape and size. He did not, however, differentiate between bodies formed from simple and compound primordia/

primordia. In both of these cases only the A type spore was present. WEHMEYER (70, p. 244) found pycnidia associated with Diaporthe galericulata, (Tul.) Sacc. in nature, which had a structure composed of more or less blackened stromatic tissue containing one or more locules. B type spores were formed in these locules. Wehmeyer stated, "The occurrence of such spores suggests the imperfect stage may be a Phomopsis, and these the Beta type of spore, which has not previously been noted".

The pycnidium tending to become chambered with more than one cavity is therefore not outside the pale of our conception of the genus Phomopsis. Surely, in certain of the species which are now known to be connected definitely with Diaporthe as the perfect stage, fruit bodies tending to become multilocular have been observed both in nature and in culture (70, p.248).

It would seem, therefore, that amongst the forms of Phomopsis on conifers, which in themselves represent only a comparatively small number of species, that we are probably dealing with species occurring in a developmental series. For this reason those species may be regarded as transitional in which the B type spore does not occur (in one instance referred to, only scantily), and in which the inner pycnidium tends to become multilocular. Until the entire authentic life-history of these so called transitional/
al/

transitional species can be determined, Sydow did not give the perfect stage of his Phomopsis Strobi, nor has the ascomycetous stage of P. Pseudotusgae or P. abietina been definitely proven), it becomes very apparent that for the present they may be regarded as belonging within the genus Phomopsis, which genus they would seem most closely to resemble. With regard to the establishment of a possible new genus for group (2), the author is very hesitant; for he is quite out of sympathy with certain tendencies in the past to erect new genera on the basis of each different grouping of characters, without a fuller knowledge of the relationships and variations of other species of the phylogenetic group in which the genus in question lies.

The above consideration becomes all the more apparent when one considers the development in culture of pycnidia arising from simple and compound primordia for the two groups (1) and (2). Fundamentally, this development was the same. In both cases the same type of simple primordium was limited by a brown outer layer. In the case of the non-B spore-forming group the primordium seemed to be smaller in size (less stromatic). In both cases, however, in the smallest primordia, a single cavity was formed; in larger primordia cavities were observed to arise at various points which fused to form an irregular cavity with a more or less continuous convoluted hymenium; or fusion was incomplete and the cavities remained separate. In both groups this multilocular condition of the/

the fruit body became very noticeable where many contiguous primordia had fused to form a solid mass of tissue. ARCHER (1, p. 23) described this same multiple cavity formation in the developmental study of Phomopsis coneglanensis, Trav.

In classifying the conifer Phomopsis advantage was taken, of differences in the number of loculi formed, in simple pycnidia derived from single primordia. In further classifying these two groups, the most useful and certain basis for a natural classification, was the type, shape and size of the conidia. The form of the spore apices, their dorsiventrality and the presence of peculiarly shaped spores, were also morphological characters which were of value in separating the forms into specific groups.

Physiological characters, such as growth culture characteristics, host adaptations, pathogenicity, + also aided in supplementing morphological characters used in segregating species.

Amongst organisms possessing so few morphological characters which could be utilised for the purpose of species differentiation, it is quite apparent how very important the physiological factor becomes in aiding the morphological separation of forms. In this study a mere cultural difference alone, was not considered in itself as being sufficient to separate a species, nor was a biometrical difference alone, considered sufficient. Fungi showing either of such differences/

differences, without exhibiting other essential differences, can only be regarded as forms of a given species. On the other hand if several good morphological differences existed, e.g., shape and size of spores; presence or absence of different spore types. together with cultural differences, then the writer, acting in accordance with the general tendency for the naming of fungus species in the past, has classified accordingly his forms as species, - always provisionally however; for if it can be shown later that these so-called species are merely after all, forms in a series, the missing connecting links of which have not at present been found and described, he is quite willing that the specific status which he has assigned to these organisms should be modified in accordance with their natural relationships.

DICHOTOMOUS KEY TO THE SPECIES OF PHOMOPSIS ON
CONIFERS.

Pycnidia ectostromatic, with or without a dark line circumscribing the stroma, simple or compound, variable in size, subspherical, cone shaped, truncate, with a flattened base, seated upon and incorporated with the cells of the outer cortex, becoming semi-erumpent; simple pycnidium formed from a single primordium, inner pycnidium in some with a unilocular cavity, tending to form in one plane, as a lens-shaped subspherical or conical chamber with a thickened pseudoparenchymatous buffer layer above; in others, the inner pycnidium with several/^{small} subspherical chambers occupying the greater portion of the inner structure, and tending to fuse to form an irregular unilocular cavity with incomplete septa, hymenium irregularly convoluted, lining the cavity; closed fruit bodies with a definite ostiole, or breaking open irregularly; compound pycnidia, formed by the fusion of adjacent primordia, cavities fusing in one plane to form an elongate irregular, convoluted cavity, or tuberculate, with many subglobose chambers, Spores of three types, A, B, and the intermediate; A and B types generally occurring, occasionally only the A type produced; A spores, unicellular, hyaline, oblong, elliptic to fusoid, commonly with two guttules; B spores, unicellular. hyaline, long-cylindrical, filamentous, straight, arcuate or hamate, with numerous minute guttules; intermediate spores intergrading between/

between the A and the B spores. Sporophores variable in size, abbreviated or longer processes, flexuous, subulate, arising from the hymenium lining the chambers. Almost universally the imperfect stage of species of the Diaporthe group.....

.....PHOMOPSIS.

Page.

a. Simple pycnidia very stromatic, unilocular, loculus formed in one plane, frequently with protrusions from the side walls with a thickened layer of tissue above.

b. Both A and B type spores present.

c. A spores generally oblong elliptic with obtuse extremities, or one extremity acute, B spores straight or crooked at one end like a walking stick; perfect stage, Diaporthe conorum Neissl; widely distributed, 14 coniferous host genera..Diaporthe conorum.
(P.occulta).

p. 49.
p. 108.

cc. A spores generally elliptic with subacute extremities; often slightly constricted at the medium part of the spore; B spores, flexuous, tending to be straight; perfect stage unknown; parasite on Cupressaceae...P. juniperovora.

p. 137.

ccc. A spores generally fusiform; B spores, much curved, horse shoe shaped; perfect stage unknown; hosts, Pinus, Pseudotsuga, Picea..... P.conorum.

p. 155.

aa./

- aa. Simple pycnidia less stromatic;
small primordia, unilocular,
loculus consisting of a single
subspherical chamber; larger
primordia multilocular,
chambers tending to fuse and
become unilocular with par-
tial septa; thickened
layer of tissue above not so
pronounced.
- b. Both A and B spores present.
- c. A spores generally oblong-
elliptic; B spores,
long-cylindric, slightly
curved: perfect stage
unknown; only known
host, Abies lasiocarpa.....P.Montanensis. p. 173.
- bb. Only A spores present.
- c. Spores generally oblong-
elliptic; small,
irregularities in shape
not noted, nor presence
of elongated cylindric
A spore type; perfect
stage not known; hosts
Pinus, Abies.....P.Strobi. p. 183.
- cc. Spores generally elliptic-
fusoid.
- d. Spores small, irregu-
larities in shape not
noted; presence of
elongate cylindric
spore type; perfect
stage not known;
hosts, Pseudotsugae,
Larix, Cedrus,
Sequoia, Abies; para-
site on Douglas fir... P.Pseudotsugae. p. 190.
- dd. Spores large, fusiform,
occasionally with
irregularities in shape;
perfect stage not known;
only known host, Abies
pectinata..... P.abietina. p. 201.
- ccc./

ccc Spores generally irregular fusiform, with protuberances, becoming three or four sided with salient angles; large; perfect stage unknown; only known host, Abies grandis.....

P.Boycei.

p. 213.

1. Diaporthe conorum (Desm.) Niessl.

(Phomopsis occulta, Trav).

Ann. Sc. VI, 1846, p. 76; Hedwigia XV,
Syll.
1876, p. 1; Sacc./Fung. I, 1882, p. 647.

Syn.: - Sphaeria conorum, Desm., (1846)

- Valsa occulta, Fuck. Fungi rhen. - n.622,
(1863).
- Sphaeria conorum, West., nec Desm. (teste Niessl,
Hedw., 1876).
- Sph. strobilicola, Lib. ined.
- Diaporthe occulta, (Fuck.) Nke. Pyr.germ., p.
266, (1870).
- D. occulta f. ramulorum, Sicc. Roum. Fung.gall.
n.5427. (1890).
- D. pitya, Sacc. Fung. ven., Ser.IV, p.7, (1875).
- D. pinophylla, Pl. et Phil., Grev. IV, p.124,
(1876)
- D. pinicola, Hazsl. (1892) (Sacc. Syll.Fung.
XIV, p.546, 1899).
- D. conigena, Feltg. (1903) (Sacc. Syll. Fung.
XVII, p.674 1905).
- D. Thujana, Petrak, Ann. Mycol., XIX, p.50
(1921).

HISTORY OF THE FUNGUS.

Diaporthe conorum, (Desm.) Niessl was originally described^{by} DESMAZIÈRES (1846) (11) on the cone scales of Pinus sylvestris in France as Sphaeria conorum. In his description he states that ascospores were 8 μ long (1/120 mm.).

NISSL (42) in 1876 called attention to specimen, No. 913 of Sphaeria conorum on cone scales of Picea excelsa in Westendorp's herbarium. Niessl stated that this specimen was identical with D. occulta, (Fuck.) Nke. (1863). However, he was of the opinion that the name given by Fuckel was not to be changed; for it was highly probable that the fungus in Westendorp's herbarium was not the same as the one Desmazières had originally described as Sph. conorum. Upon the basis of the difference in length of the perithecial ostiole, and the different host substratum, Niessl concluded that Desmazières must have had before him another fungus distinct from Fuckel's D. occulta (19, 43). He therefore made the new combination D. conorum, (Desm.), differentiating this fungus on cones of Pinus sylvestris from D. occulta, Nke. (Syn: Sphaeria conorum, West., nec Desm., teste Niessl), on cones of Picea excelsa.


SACCARDO (54) in his description of Phoma (Diaporthe) conorum, Sacc. (1882), attributed this fungus to be the imperfect stage of D. conorum.

In 1906, TRAVERSO (66, p. 221) gave a description/

description of Diaporthe occulta on the cone scales of Norway spruce and Italian cypress in Italy. He also described D. pitya, Sacc. (66, p.284) as occurring on dead branches of Picea excelsa and Abies pectinata. His description, however, in no wise set these two forms apart as distinct species. The greatest difference in structure seemed to consist chiefly in the length of the perithecial ostiole. Traverso apparently regarded one species as a stem form and the other as inhabiting cones. He did not mention a fungus occurring in Italy corresponding with D. conorum as described by Desmazières.

In 1906, von ^{II}HÖHNEL (32) commented upon the type of Diaporthe conigena, Feltg. (Vorstud. Pilz. Lux., Nachtr. III, 1903, p. 136; Sacc. Syll. Fung. XVII, p. 674) in Feltgens herbarium as being fully identical with D. occulta. Since he was unable to locate the type of D. pinastri, Feltg. (Vorstud. Pilz. Lux., Nachtr. II, 1899, p. 126; Sacc. Syll. Fung. XIV, p. 546) he stated that the species should be struck out. Later, in 1917, von ^{II}HÖHNEL (33) suggested that another species of Diaporthe/^{D.}pitya, was to be considered only as a form of D. occulta (Pl. I. Fig. 1).

A perusal of the foregoing historical data, indicated that there was considerable doubt regarding the true identity of Desmazières Diaporthe (Sphaeria) conorum. Apparently there existed a very close relationship between this fungus and other species of Diaporthe, among which D. conigena and D. pitya had already been recognised as forms of D. occulta.



EXAMINATION OF EXSICCATI.

When the author commenced his investigation of the identity of Diaporthe conorum, (Desm.) Niessl, an investigation which was particularly pertinent because of the reputed connection between this fungus and the imperfect stage, Phomopsis (Phoma) conorum, (Sacc.) Died. (54 II, p. 615) on the cones of spruce, he obtained exsiccati from Kew Herbarium of this ascomycete together with exsiccati of D. occulta, Fuck., Nke. which included the type (Valsa occulta, Fuck., Fung. rhen. n. 622).

A study of the specimens from Kew of these two Diaporthe species impressed the writer with the fact that he was very probably dealing with forms of the same fungus species. The exsiccati all appeared to be Diaporthe occulta, and were in morphological agreement with the type specimen of Diaporthe occulta.

It was here interesting to note that Grove queried the identification of certain specimens of Diaporthe conorum, for, on the Kew Herbarium sheets of this species were to be found annotations by him; specimen No. 913, Sphaeria conorum, Desm., on the cone scales of Picea excelsa, (ex Herb. Hort. Bot. Belg.), - "According to Niessl, this is Diaporthe occulta (Fck.) Nits."; specimen No. 1773, Sp. conorum, Desm., on cone scales of Pinus sylvestris, Ser. I. (1825-1851), Desm. Crypt. France, - "?"

As a result of the examination of the Kew exsiccati of Diaporthe conorum and D. occulta a discrepancy/

discrepancy in Desmazières' description so far as the measurements of the ascospores of this fungus were concerned was strongly suspected. Accordingly, in the event that a type specimen could not be found agreeing with the original description of Desmazières' species, the author felt justified in amending the description given by Desmazières for D. conorum, and placing the name D. occulta (1863) as a synonym under this older species (1846).

An investigation, therefore, of the type specimen of Diaporthe conorum became highly desirable, (1) to clear up the identity of the fungus in order that its life-history might be comprehensively studied and interpreted from freshly collected, living forms of the species and (2) to clarify the confusion existant in the literature concerning this fungus and closely related forms.

Desmazières was known to have deposited a collection of fungi agreeing with his catalogue at the Laboratoire de Botanique, Lille, France. A specimen of Diaporthe conorum on the cone scales of Pinus sylvestris was obtained from this Herbarium which agreed with Desmazières' fungus list. With very little doubt, this exsiccatus can be regarded as a type specimen for that species. An excerpt from a letter written by Dr. M. Hocquette, Director of the Laboratory to Dr. Malcolm Wilson, confirmed this point:

"Le specimen de Sphaeria conorum que je vous ai communiqué provient de l'herbier personnel de Desmazières et certainement il n'y a pas lieu de penser à une erreur car l'échantillon correspond exactement/

exactement à celui indiqué dans le catalogue composé par Desmazières lui-même.

L'étiquette ne porte aucune indication de date ni de localité."

An examination of the Lille type of Diaporthe conorum showed that the stromatic structure of the fungus, was very similar to that recognised as characteristic for forms of Diaporthe occulta and fresh material of a species which had been recognised as D. pitya on Douglas fir collected in Scotland.

Enough spores were secured from the type to calculate its biometrical constants for comparison with other Diaporthe forms. The measurement of 100 spores indicated clearly that Desmazières had made an error in his calculations of the spore size of the species.

The extreme spore range was found to be 9.3-13.3 x 2.5-4.0 μ , and the common range, 9.6-12.4 x 2.8-3.7 μ .

In shape the ascospores were identical with those to be found in exsiccati of D. occulta. (Plate III.)

Inasmuch as Desmazières' fungus agreed with Fuckel's type of Diaporthe occulta, and with other exsiccati identified as D. occulta, it was evident that the two species were identical and an amended description for D. conorum became necessary. Accordingly D. occulta became a synonym of D. conorum.

Associated with the problem of the determination of the identity of Diaporthe conorum and D. occulta, was the additional problem of solving the relationships of several other species of Diaporthe on conifers which according to their descriptions in the/

the literature were recognised as closely resembling D. conorum. These species of Diaporthe were as follows:-

Diaporthe pitya, Sacc. (1873). Fung. ven., ser. IV, 1875, p. 7; Sacc. Syll. Fung. I, p. 689.
D. pinophylla, Pl. et Phil. (1876), Grev. IV, p. 124.
D. pinicola, Hazsl. (18-2) Math. e. term. Kozlem. XXV, p. 200;
Sacc. Syll. Fung. XIV, p. 546.
D. Thujana, Petrak (1921) Ann. Mycol. XIX, p. 50.

A specimen of Diaporthe pitya, (Sacc.) n. 93, Mycoth. ital. (1897) on Picea excelsa was examined at the British Museum (Natural History) London. This specimen proved to be D. conorum, as did exsicatus, D. pinophylla, Pl. and Ph. n. 37, on needles, Pinus sylvestris, Plowright Sph. Britt. III, from Kew Herbarium.

The author was unable to obtain the original type of Petrak's new species Diaporthe Thujana for examination, but from the description (44) given he could probably safely say that this Diaporthe is only a form of D. conorum. When Petrak provisionally described his species collected on Thuja sp. he regarded it as the perfect stage of Phomopsis Thujae, Died. P. Thujae is now known to be the imperfect stage of D. conorum (see p. 119).

The type of Diaporthe pinicola on Pinus sylvestris in Hungary can also probably be regarded as a form of D. conorum, although this statement is made without the author having seen the actual type specimen of this fungus.

von Höhnel/

von Höhnelt, as has been stated in the previous section, had already expressed his opinion concerning the identity of Diaporthe pinicola and D. pitya with D. occulta.

A survey of the foregoing impresses one with the comparatively large number of forms, previously recognised as distinct species, which may now be regarded as forms of a single species. In the research amongst the exsiccati of Diaporthe conorum and related forms, the author examined the following exsiccati and found them all to be that species:-

- Sphaeria conorum, Desm., on Pinus sylvestris, type deposited by Desmazières at the Lab. d. Bot., Lille.
- no. 1773. Sphaeria conorum, Desm., Pinus sylvestris cones, Crypt. France, Ser. I (1825-1881); (Kew Herb.).
- " 913. Sphaeria conorum, on Picea excelsa cones, ex Herb. Hort. Bot. Belg. (Kew Herb.).
- " 1563. D. conorum, (Desm.) Neissl. P. excelsa cones, Roum. Fgi. Gall. Reliq. Lb. (Kew Herb.).
- Sph. conorum, (Desm. 1773), on P. sylvestris, Herb. Berk. (Kew Herb.).
- Sph. conorum, Desm., on P. excelsa, coll. Lb., (Jard. d. bot., Bruxelles).
- D. conorum, (Desm.) Niessl, coll. ? Lb., on P. excelsa cones, (Sacc. Herb., Padua).
- no. 293. D. conorum, Fuck., coll. Lb., on P. excelsa cones, (Sacc. Herb. Padua).
- " 272 D. conorum, coll. Lb., on P. excelsa cones, (Sacc. Herb. Padua).
- Sph. strobilicola, Lib., Herb. Jard. bot. Bruxelles, (Kew Herb.).
- no. 622. Valsa occulta, Fuck. on P. excelsa cones, Fungi rhenani, (Kew Herb.).
- " 669. Diaporthe occulta, (Fuck.) Nke., Roum. Fgi. Gall., (Kew Herb.).
- " 5427. D. occulta f. ramulorum, Roum. Fgi. Gall., (Kew Herb.).
- " 93. D. pitya, Sacc., on P. excelsa stem, Mycoth. ital. (Brit. Mus. London).
- " 37. D. pinophylla, Pl. et Phil., P. sylvestris needles Plowright Sph. Brit. III. (Kew Herb.).

A biometric study has been made of seven of the forms listed above including the type. The size of spores of the species Diaporthe conorum an amended description of which follows, and their variation within a specific range will be discussed in the sections dealing with the size of ascospores in nature and culture.

DESCRIPTION OF THE FUNGUS.

Diaporthe conorum (Desm.) Niessl descr. ement.

Stromata effuse, blackening the surface, occurring in the upper bark tissues of the stems commonly in the periderm but extending throughout the cortex into the wood (55, Pl., 1277) also on leaves or cone scales; stroma circumscribed by a meandering black line; perithecia immersed in cortex, separate or aggregate, appearing in a circle in groups of three to ten; sphaeroidal, .25-.5 mm. diam., somewhat thin walled, olivaceous, pseudoparenchymatous; ostiole or neck consisting of elongated cells, ostioles scarcely projecting beyond the epidermis through which they become erumpent, or greatly elongate (Pl.IV Fig,1) slender, sinuous, flexuous, frequently curved; apex slightly drawn out; asci with very delicate walls, cylindrical, or tending to become spindle shaped with reduced extremities, the wall thickened both at the base and at the apex; the latter with a ring of protoplasm set down within the thickened tip (Plate 40-55 x 5-8 μ); asci readily released, filling the cavity of the fruit body with a whitish perithecial centrum of asci and liberated ascospores; asci with eight spores; paraphyses not observed; ascospores hyaline, uniseptate, distichous or rarely monostichous, blunt, varying from straight, long elliptic, non-constricted, to broad elliptic, elliptic-fusoid, constricted/

constricted, slightly curved (Pl. II Fig. 1); generally with four guttules; measurement of 700 spores from exsiccati, including the type specimen (Lille) showed an extreme range of 9.3 -16.1 x 2.5-5 μ , commonly 9.6 - 14.3 x 2.8-4.3 μ .

The imperfect stage, culturally proven, is Phomopsis occulta, Trav. (see p.103).

Hab. According to Saccardo and Traverso on cone scales of Picea excelsa, Pinus sylvestris and Cupressus sempervirens (Syn: D. occulta) in France, Germany, Belgium, Great Britain and Italy. The following citations of the occurrence of the species have been made by the following authorities:-

Traverso (Syn: D. pitya) on dead branches of P. excelsa and Abies pectinata in Italy; Plowright and Phillips (Syn: D. pinophylla) on needles of P. sylvestris in England; Hazslinszky (Syn: D. pinicola) on dead branches of P. sylvestris in Hungary; Lind (Syn: D. pitya) on dead branches of P. excelsa in Denmark; Petrak (Syn: D. Thujana) on dead branches of Thuja sp.; Wilson (Syn: D. pitya) on dead branches of Pseudotsuga Douglasii and Abies grandis in Scotland.

The fungus has been collected or identified by Hahn on dead branches of Pseudotsuga Douglasii, Abies pectinata and Picea Sitchensis, and on cone scales of Picea excelsa and Larix europaea. He has identified the imperfect stage, Phomopsis occulta on fourteen coniferous host genera (see p. 113) in North America and Europe.

SIZE OF ASCOSPORES OF D. CONORUM IN NATURE AND CULTURE

Variation of Spores in Nature.

A comparison of the biometric constants of 100 ascospores of the type of Diaporthe occulta, Nke. (Valsa occulta, Fuck., Fung. rhen. no.622) with those of 100 spores each, of the following exsicatti:-

no. 1773, Sphaeria conorum, Desm.Crypt.France,Ser.1.
(1825-1851) (Kew Herb.).
" 913. Sphaeria conorum, ex Herb.Hort.Bot.Belg.
(Kew Herb.).
-----, Sphaeria strobilicola, Lib.,ex Herb.Jard.
bot. Bruxelles (Kew Herb.).
no. 669. Diaporthe occulta, Roum. Fgi. Gall.(" " ").

showed that a significant difference did not occur between any one of these four exsicatti and Fuckel's type.

The significant difference was (51) based upon the relation of the difference of the means of the type specimen and of the exsicatus being compared, to the probable error of the difference.

In Tables I and II, exsicati of Sphaeria conorum, Sph. strobilicola and Diaporthe occulta are compared with the type of the last named species:

Table 1/

(TABLE I) BIOMETRIC CONSTANTS of ASCOSPORES of DIAPORTHE (VALSA) OCCULTA (TYPE) SPHAERIA CONORUM SPH. STROBILICOLA and DIAPORTHE OCCULTA.

LENGTH

POPULATION	NO. SPORES	M	SIGNIF. of MEAN DIFFERENCES SIGNIF. from	STANDARD DEVIATION	COEFFICIENT of VARIABILITY
<u>Valsa occulta</u> (type)	100	12.9 ±.058		.852 ±.041	6.60 ±.329
Ex. 1773 (1) <u>Sph. conorum</u>	100	12.3 ±.054	none	.805 ±.038	6.55 ±.314
Ex. 913 <u>Sph. conorum</u>	100	12.9 ±.062	none	.921 ±.044	7.14 ±.342
Ex <u>Sph. Strobilicola</u>	100	12.8 ±.055	none	.821 ±.039	6.41 ±.307
Ex. 669 <u>Diaporthe occulta</u>	100	12.4 ±.052	none	.776 ±.037	6.26 ±.30

(1) On cone scales Pinus sylvestris; other specimens on cone scales

Picea excelsa.

(TABLE II) BIOMETRIC CONSTANTS of ASCOSPORES of DIAPORTHE (VALSA)
OCCULTA (TYPE), SPH. CONORUM, SPH. STROBILICOLA, D. OCCULTA
 WIDTH

POPULATION	NO SPORES	M u	SIGNIF. of MEAN DIFFERENCES SIGNIF. from	STANDARD DEVIATION	COEFFICIENT of VARIABILITY
<u>Valsa occulta</u> (type)	100	3.5 ±.021		±.305 ±.015	8.72 ±.419
Ex 1773 <u>Sph. conorum</u>	100	3.4 ±.022	none	±.328 ±.016	9.65 ±.465
Ex 913 <u>Sph. conorum</u>	100	3.6 ±.031	none	±.453 ±.022	12.58 ±.610
Ex <u>Sph. strobilicola</u>	100	3.5 ±.024	none	±.354 ±.015	10.10 ±.487
Ex 669 <u>Diaporthe occulta</u>	100	3.5 ±.028	none	±.415 ±.020	11.86 ±.582

Morphologically, at least, the foregoing forms originally identified as Diaporthe conorum and D. occulta were identical with the type of D. occulta; for morphological characters other than size, such as shape of the ascospores (PL. III, Figs. 1-6) and appearance of the stroma were likewise in agreement. It is here interesting to observe the modes and the extreme and common ranges of the ascospores of D. (Valsa) occulta, type, and the exsiccati compared biometrically. TABLE III contains ^{e, e}this data. The similarity between the spore size ranges of ascospores on spruce and Scots pine cones is very striking. Variation in size is indicated.

(TABLE III) THE MODES, COMMON and EXTREME SIZE RANGES of ASCOSPORES of the TYPE of D. OCCULTA and EXSICCATI BEING COMPARED

EXSICCATUS	NO. SPORES	MODE, μ .		COMMON RANGE length by width, μ	EXTREME RANGE length x width, μ
		Length	width		
<u>Valsa occulta</u> (type)	100	12.4	3.7	11.5-14.3x3.1-4.3	11.2-14.9 x 2.8-4.7
Ex 1773 <u>Sph. conorum</u>	100	12.1	3.4	10.9-13.6 x 2.8-4.0	10.5-14.0 x 2.5-4.3
Ex 913 <u>Sph. conorum</u>	100	13.0	3.7	11.5-14.3 x 3.1-4.3	10.9-16.1 x 2.8-5.0
Ex <u>Sph. strobilicoides</u>	100	12.4	3.7	11.5-14.3 x 3.1-4.0	11.2-14.9 x 3.1-4.3
Ex 669 <u>Disporthe occulta</u>	100	12.4	3.4	11.2-13.7 x 3.1-4.3	10.5-14.0 x 3.1-4.7
Total Range	500			10.9-14.3 x 2.8-4.3	10.5-16.1 x 2.8-5.0

When the exsiccatus of Diaporthe (Sphaeria) conorum (which is considered the type specimen), was obtained from Desmazières herbarium at Lille, and examined, preliminary observations showed that while the ascospores of this type specimen on Pinus sylvestris agreed in shape with those of the type specimen of D. (valsa) occulta on Picea excelsa and were produced in a similar entostroma, they nevertheless appeared to be somewhat smaller in size, both in length and in width. The biometric constants of a population of 100 ascospores of D. conorum (Lille type) are compared with those of the type D. occulta in (TABLE IV). A significant difference between spore populations of the two types is indicated.

TABLE/

(TABLE IV) BIOMETRIC CONSTANTS OF ASCOSPORES OF D. (SPHAERIA) CONORUM (TYPE)
 COMPARED WITH D. (VALSA) OCCULTA (TYPE)
 LENGTH

POPULATION	NO SPORES	M u	SIGNIF. OF MEAN DIFFERENCES SIGNIF. from	STANDARD DEVIATION	COEFFICIENT of VARIABILITY
<u>D. (Sphaeria)</u> <u>conorum</u>	100	11.2 ±.061		.91 ±.043	8.13 ±.390
<u>D. (Valsa)</u> <u>occulta</u>	100	12.9 ±.058	Signif. from	.852 ±.041	6.60 ±.329
			WIDTH		
<u>D. conorum</u>	100	3.1 ±.017		.246 ±.012	7.93 ±.381
<u>D. occulta</u>	100	3.5 ±.021	Signif. from	.305 ±.015	8.72 ±.419

If the modes, common and extreme ascospore size ranges are compared,
 this difference in size expressed above in the biometrical tables, is brought
 out in (TABLE V)

(TABLE V) THE MODES, COMMON and EXTREME SIZE RANGES of ASCOSPORES of the TYPES of D. CONORUM and D. OCCULTA, COMPARED with EACH OTHER, and with the RANGE of 500 SPORES GIVEN in TABLE III.

EXSICCATUS	NO SPORES	MODE, μ		COMMON RANGE LENGTH X WIDTH, μ	EXTREME RANGE LENGTH X WIDTH
<u>D. (Sphaeria) conorum</u>	100	10.9	3.1	9.6-12.4 x 2.8-3.7	9.3-13.3 x 2.5-4.0
<u>D. (Valsa) occulta</u>	100	12.4	3.7	11.5-14.3 x 3.1-4.3	11.2-14.9 x 2.8-4.7
Total average	500	(See TABLE III)		10.9-14.3 x 2.8-4.3	10.5-16.1 x 2.8-5.0

When the modes, common and extreme ascospore size ranges of the two types are compared, the significant difference expressed in TABLE IV, is emphasized again in TABLE V.

It will be noted however, that the ascospore range of the type, Diaporthe conorum, closely overlaps that of the range indicated by the measurement of the 500 spores (including 100 spores of the type, D. occulta) given in TABLE III. Furthermore the range of the type, D. conorum, very closely overlaps that of exsiccatus, no. 1773, Sphaeria conorum, Desm. Crypt. France, Sec 1.(1825-1851) (See TABLE III) on the same host. The length mode of the former is approximately at 11u, the mode of the latter, at 12u.

Despite the difference in spore size shown by the type of Diaporthe conorum, the very fact that this fungus otherwise resembled morphologically, the type of D. occulta, and the exsiccati compared with the latter fungus, brought the author to the consideration that he was dealing very probably, with the same species. The spore size of the type of D. conorum therefore, could be interpreted as a variation within the size range of the species itself, (the specific range), and this type represented a somewhat lower extension of the range, as that expressed by the measurement of 500 spores (see TABLE/

TABLE III) of D. occulta and exsiccati resembling this fungus.

To corroborate this line of reasoning, an interesting specimen was measured at this time which agreed closely in size with the type of Diaporthe conorum, but showed a significant difference with the type of D. occulta. This particular exsiccatus, no.5427, D. occulta f. ramulorum, Roum. Fgi. Gall. (at Kew Herb.) had been collected on the stems and not on the cones of Picea excelsa. Morphologically it agreed with both types in characters other than size. In (TABLE VI) the biometrical constants of D. occulta f. ramulorum, are compared with the two types. A significant difference in length is indicated with the D. occulta, on the same host, but collected on cone scales:

TABLE/

(TABLE VI) BIOMETRICAL CONSTANTS of D. OCCULTA f. RAMULORUM COMPARED
with the TYPES of D. CONORUM and D. OCCULTA

LENGTH

POPULATION	NO SPORES	M u	SIGNIF. of MEAN DIFFERENCES SIGNIF. from	STANDARD DEVIATION	COEFFICIENT of VARIABILITY
Ex. 5427 <u>D. occulta</u> f. <u>ramulorum</u>	100	11.9 ±.061		.905 ±.043	7.61 ±.333
<u>D. conorum</u> (type)	100	11.2 ±.061	none	+.910 -.043	8.13 ±.390
<u>D. occulta</u> (type)	100	12.9 ±.058	Signif. from	+.852 -.041	6.60 ±.329
WIDTH					
Ex. 5427 <u>D. occulta</u> f. <u>ramulorum</u>	100	3.3 ±.022		+.329 -.016	9.97 ±.480
<u>D. conorum</u> (type)	100	3.1 ±.017	none	+.246 -.012	7.93 ±.381
<u>D. occulta</u> (type)	100	3.5 ±.021	none	+.305 +.015	8.72 ±.419

Diaporthe occulta f. ramulorum (100 spores) showed a spore range with a lower size limit equal to that of the type of D. conorum (see TABLE V), - common range 10.5-13.3 x 2.8-4.0 u, extreme range, 9.3-14.3 x 2.8-4.3 u. Undoubtedly the foregoing Diaporthe forms must be regarded all as the same species. The difference in size, which occurred between various forms, and between certain of these forms and the original type specimen (D. conorum) can be interpreted in terms of variability in size within the range for the species.

Freshly collected ascospore material of D. conorum from different conifer hosts in Scotland and England, when measured and treated biometrically, showed significant differences among themselves, together with a certain amount of variation in size. Significant differences, between certain of these forms and the type of D. conorum were likewise observed. However it was noted that whatever variations did occur these were confined within the aggregate extreme spore range determined for the type of D. conorum and the exsiccati (see TABLES III & V) morphologically resembling that type.

The/

The following forms of D. conorum were studied hiometrically:

<u>Pseudotsuga</u> <u>Douglasii</u>	10 collections,	Scotland
<u>Pseudotsuga</u> <u>Douglasii</u>	43931, 1	" (cultured) "
<u>Picea</u> <u>Sitchensis</u>	43979, 1	" (")England
<u>P. excelsa</u>	43984, 1	" (")Scotland
<u>Larix</u> <u>Europaea</u>	43986, 1	" "

TABLE VII presents the biometrical constants of freshly collected forms of D. conorum compared with the type specimen of that fungus. The significant differences are indicated.

TABLE/

(TABLE VII) BIOMETRIC CONSTANTS of FORMS of DIAPORTHE CONORUM
COLLECTED in NATURE and COMPARED with the TYPE.

LENGTH

POPULATION	NO. SPORES	M \bar{x}	SIGNIF. of MEAN DIFFERENCES SIGNIF. from	STANDARD DEVIATION	COEFFICIENT of VARIABILITY
<u>D. conorum</u> (type)	100	11.2 ± .061		.910 ± .043	8.13 ± .390
<u>Pseudotsuga</u> <u>Douglasii</u> form (10 collections)	100	11.7 ± .058	none	.853 ± .041	7.30 ± .350
No. 43931 <u>P. Douglasii</u>	100	12.9 ± .067	Signif. from	.996 ± .048	7.72 ± .370
No. 43979 <u>Picea Sitchensis</u>	100	12.5 ± .052	Signif. from	.775 ± .037	6.20 ± .297
No. 43984 <u>Picea excelsa</u>	100	11.4 ± .064	none	.943 ± .045	8.29 ± .398
No. 43986 <u>Larix europaea</u>	100	11.9 ± .048	none	.710 ± .034	5.94 ± .284

WIDTH

<u>D. conorum</u> (type)	100	3.1 + .017 -		.246 + .012 -	7.93 + .381 -
<u>Pseudotsuga</u> <u>Douglasii</u> form (10 collections)	100	3.3 + .023 -	none	.341 + .016 -	10.33 + .499 -
No. 43931 <u>P. Douglasii</u>	100	3.3 + .021 -	none	.317 + .015 -	9.6 + .462 -
No. 43979 <u>Picea Sitchensis</u>	100	3.5 + .021 -	Signif. from	.304 + .015 -	8.69 + .418 -
No. 43984 <u>Picea excelsa</u>	100	3.0 + .015 -	none	.224 + .011 -	7.47 + .358 -
No. 43986 <u>Larix europaea</u>	100	3.2 + .020 -	none	.297 + .014 -	9.27 + .446 -

Where significant differences in length are indicated in TABLE VII it is here very interesting to state that culturally, the growth characteristics of No. 43931 (Pseudotsuga Douglasii) were identical with those of No. 43984 (Picea excelsa). The former fungus was isolated from a stem, the latter from a cone scale. Furthermore, the culture growth characteristics of No. 43979 (Sitka spruce) were identical with the two forms just mentioned. In the above considerations we appear to be dealing with the factor of variability. Had differences in culture characteristics been found, we would have strongly suspected physiological strains or forms of the fungus species D. conorum. The total common and extreme spore size ranges of ascospores of the species is given in TABLE VIII for spores from exsiccati, and for spores freshly collected in nature from three generic hosts:

TABLE/

(TABLE VIII) COMMON and EXTREME SIZE RANGES of ASCOSPORES of
D. CONORUM MEASURED from EXSICCATI and from FRESHLY
 COLLECTED SPECIMENS.

EXSICCATI OR NATURE	NO SPORES	COMMON RANGE LENGTH X WIDTH, μ	EXTREME RANGE LENGTH X WIDTH, μ
Type and 6 exsiccati	700	9.6-14.3 x 2.8-4.3	9.3-16.1 x 2.5-5.0
14 collections, Nat.	500	9.6-14.6 x 2.8-4.3	9.3-15.2 x 2.5-4.3

It is very evident that the spore size range of the two
 groups being compared, is very close.

In connection with the discussion of the spore size range of Diaporthe conorum, a range which was indicated as being specific, a consideration of the identity of D. Taxi Oud. et Dest. (Sacc. Syll. Fung. XIV, P. 546) came up. This species was described on Taxus as having spores, 18-21 x 9, μ , asci 116 μ . On the basis of its extremely larger size, this form is very probably a distinct species. The writer did not examine the type specimen of this fungus. Forms of Phomopsis occulta (D. conorum) on Taxus, however, have been studied. These agreed morphologically and culturally with forms of the same species on Picea, Pinus, Pseudotsuga, Larix, etc.

Variation/

Variation of Ascospores in Culture.

In order to test further the variability of size of ascospores of Diaporthe conorum within the specific range determined for that species as the result of the biometric study of 1200 spores from exsiccati and from nature, certain forms of the species were isolated in pure culture, and the spores produced on artificial ^{As} substrata were likewise investigated. Comparisons were made between the spores produced in culture with those formed under natural conditions.

TABLE IX presents the biometric constants for length, and breadth respectively of spores of a form of Diaporthe conorum (No.43931) in a mono-ascospore culture isolated from Pseudotsuga Douglasii. The culture medium used was a sterile Douglas fir (P. Douglasii) twig.

Biometrically a significant difference was not found.

TABLE/

(TABLE IX) BIOMETRIC CONSTANTS of ASCOSPORES of D. CONORUM NO. 43931
on PSEUDOTSUGA DOUGLASI in NATURE and CULTURE.

POPULATION	NO SPORES	\bar{x}	LENGTH		STANDARD DEVIATION	COEFFICIENT of VARIABILITY
			SIGNIF. of MEAN DIFFERENCES SIGNIF. from			
Nature	100	12.9 $\pm .067$.996 $\pm .048$	7.72 $\pm .370$
Culture	100	12.5 $\pm .053$	none		.786 $\pm .038$	6.29 $\pm .301$
WIDTH						
Nature	100	3.3 $\pm .021$.317 $\pm .015$	9.6 $\pm .462$
Culture	100	3.2 $\pm .019$	none		.281 $\pm .013$	8.78 $\pm .422$

Nor did a significant difference exist between the spores of a form of D. conorum isolated from Sitka Spruce, No. 43979, and spores produced in culture on hard oat agar and a sterile Ulmus twig, respectively. (TABLE X)

(TABLE X) BIOMETRIC CONSTANTS of ASCOSPORES of D. CONORUM NO. 43979
on PICEA SICHENSIS in NATURE and CULTURE.

LENGTH

POPULATION	NO SPORES	M	SIGNIF. of MEAN DIFFERENCES SIGNIF. from	STANDARD DEVIATION	COEFFICIENT of VARIABILITY
Nature	100	12.5 ± .052		.775 ± .037	6.20 ± .297
Oat agar	100	12.0 ± .056	none	.832 ± .040	6.90 ± .331
<u>Ulmus</u> twig	100	11.8 ± .063	none	.934 ± .045	7.91 ± .380
WIDTH					
Nature	100	3.5 ± .021		.304 ± .015	.869 ± .418
Oat agar	100	3.4 ± .018	none	.269 ± .013	.791 ± .380
<u>Ulmus</u> twig	100	3.3 ± .024	none	.349 ± .017	10.59 ± .511

When the common and extreme spore ranges in nature and culture of the two forms of D. conorum Nos. 43931, 43979 were compared with the spore size range of the species (TABLE XI), it was found that while very slight variation in spore size occurred, this variation was quite within the confines of the specific range. There existed a close agreement between the size of spores produced in nature, and those produced in culture.

TABLE/

(TABLE XI) THE COMMON and EXTREME SIZE RANGES of SPORES of D. GONORUM PRODUCED in NATURE and CULTURE COMPARED with the RANGE of the SPECIES.

POPULATION	NO. SPORES	COMMON RANGE LENGTH X WIDTH, μ	EXTREME RANGE LENGTH X WIDTH, μ
<u>D. gonorum</u> , specific range	1200	9.6-14.6 x 2.8-4.3	9.3-16.1 x 2.5-5.0
<u>D. gonorum</u> 43931 Nature	100	11.5-14.6 x 2.8-4.0	9.3-15.2 x 2.5-4.0
<u>D. gonorum</u> 43931 Culture(1)	100	11.5-13.7 x 2.8-3.7	10.9-14.9 x 2.8-4.0
<u>D. gonorum</u> 43979 Nature	100	11.5-13.6 x 2.8-4.3	10.2-14.3 x 2.8-4.3
<u>D. gonorum</u> 43979 Culture(2)	100	10.5-13.3 x 2.8-4.0	9.9-14.3 x 2.8-4.0
<u>D. gonorum</u> 43979 Culture(3)	100	10.9-13.0 x 2.8-4.0	9.9-15.2 x 2.5-4.0

(1) Douglas fir twig; (2) Oat agar; (3) Elm twig.

In the following culture set, a form of D. conorum, No. 43937, isolated from Abies pectinata was used. Unfortunately the biometrical constants obtained from measurement of ascospores produced in culture could not be compared directly with those of the form in nature, for the reason, that the isolation culture was originally derived from a spore tendril colony. While perithecia initials were observed in the entostroma of this form in nature, mature ascospores were not available for measurement.

It will be observed after a consideration of TABLE XII that a significant difference occurred between the forms on Douglas fir and Elm with regard to length. In this instance longer spores were obtained with the silver fir form, than with the Sitka/

Sitka spruce form, No. 43979, on the same host (Elm) substratum. (SEE TABLE X) The writer is inclined to regard this variation in size of spores of No. 43937 on the Elm substratum, in terms of the inherent variability of the form itself, rather than the result of any undue influence of the host upon the organism. Variation in size of ascospores when it occurred for No. 43937, was also well within the specific range of the ^{is}Species. Second generation ascospores showed agreement in size with first generation spores, upon the same host substratum.

TABLE/

WIDTH

43937B ¹ Douglas fir	100	3.4 ±.020		.303 ±.014	8.9 ±.428
" <u>Acer</u>	100	3.2 ±.018	none	.265 ±.013	8.27 ±.397
" <u>Ulmus</u>	100	3.3 ±.018	none	.27 ±.013	8.89 ±.427
" ^{II} B Douglas fir	100	3.1 ±.020	none	.292 ±.014	9.43 ±.454

(1) Ascospores derived from mono-ascospore isolation; isolation from first generation of ascospores produced in culture.

(TABLE XII) BIOMETRIC CONSTANTS of D. CONORUM, NO. 43937, in CULTURE
on PSEUDOTSUGA DOUGLASII COMPARED with the SAME FORM with other
NATURAL MEDIA

LENGTH

POPULATION	NO. SPORES	MEAN \bar{x}	SIGNIF. of MEAN DIFFERENCES SIGNIF. from	STANDARD DEVIATION	COEFFICIENT of VARIABILITY
43937B ¹ Douglas fir	100	12.0 ±.056		.834 ±.040	6.95 ±.333
" <u>Acer</u>	100	11.9 ±.069	none	1.02 ±.049	8.57 ±.412
" <u>Ulmus</u>	100	13.6 ±.102	Signif. from	1.508 ±.072	11.09 ±.575
" <u>B Douglas</u> fir(1)	100	11.7 ±.046	none	.680 ±.032	5.81 ±.278

On the basis of the foregoing limited number of culture tests, which have more or less corroborated the evidence already secured from a study of the ascospores of Diaporthe conorum in nature, one may safely conclude that the range of size for the species is fairly constant, and that while variation may take place, this variability is within the specific range. Furthermore broad-leaved hosts appeared to have little or no effect in altering the specific size of the ascospores of forms derived from conifers.

SHAPE/

SHAPE of the ASCOSPORES in NATURE and in
CULTURE.

A careful study of the shape of the ascospores of Diaporthe conorum in exsiccati, and those obtained from freshly collected specimens of different conifer species, showed marked agreement with regard to this morphological character. This agreement in shape between spores observed in nature, also held for spores derived from cultures, particularly for ascospores produced on broad-leaved hosts (e.g. on natural media, twigs of Ulmus, Alnus and Acer) No difference in shape could be detected, despite the fact that the fungus was grown on substrata not ordinarily considered among the natural hosts of the fungus.

CULTURAL LIFE-HISTORY OF DIAPORTHE CONORUM.

Historical Review of Life-History Studies.

Diaporthe conorum was isolated in pure culture for the purpose of determining its complete life-history. The correct determination of the life-history of this fungus was highly desirable on account of the confusion existing in the literature regarding the imperfect connections of the species. A study of the synonymy of D. conorum as given on page shows at a glance certain well known species which have been previously associated in the literature with various imperfect stages. Cultural proof however, for these attributed connections have been almost entirely lacking.

Diaporthe occulta (= D. conorum) was regarded by FÜCKEL (19) as the perfect stage of a spermagonial form, the latter being first described by him (1875). Later this fungus was named Phoma occulta by SACCARDO (56, III, p. 150). Fuckel regarded the pyrenomycete as a rare fungus.

Diaporthe conorum (54; 56, III, p.150) was considered the ascomycetous stage of the imperfect fungus Phomopsis (Phoma) conorum (1882).

PETRAK (44) in describing his new species Diaporthe Thujana associated this fungus with the imperfect stage Phomopsis Thujae, Died.

SACCARDO/

SACCARDO (55, p. 126) had stated Diaporthe pitya (= D. conorum) to be the perfect stage of Phoma pitya, Sacc. (1878). WILSON (75, p. 11) in 1925, advanced reasons whereby P. pitya should not be regarded as the conidial stage of this ascomycete, and affirmed D. pitya to be the perfect stage of his own species Phomopsis Pseudotsugae; he gave cultural evidence for this connection.

Recent researches by WILSON and HAHN (77) have shown Phoma pitya to be a species other than a Phomopsis, and the fungus has been placed provisionally by them in the genus Sclerophoma.

This paper presents cultural proof that Phomopsis (Phoma) conorum is in no way connected with the life-history of Diaporthe conorum. The imperfect stage of this ascomycete is the fungus originally described by FÜCKEL (19) and named by Saccardo, Phoma occulta. The author has made a intensive study of P. Pseudotsugae but with none of the forms studied, has he been able to connect up a perfect stage; nor contrariwise, in no case was he able to obtain P. Pseudotsugae from monoascospore isolation cultures of D. conorum.

Culture/

Culture Growth Characteristics.

Ascospores of Diaporthe conorum freshly collected were found to germinate readily within 24 hours at ordinary room temperature either in sterile water, or in spore dilutions upon the agar surface of a sugar-corn meal agar plate. This latter medium was used entirely for single ascospore isolation purposes. The spores produced germ tubes generally towards the extremities of the spore bodies. Germ tubes were observed to originate from one cell only, or from both cells.

The fungus grew rapidly on all the media which were experimented with, namely oat, Leonian's, and sugar-corn meal agars. Culture growth characteristics showed close agreement with characteristics produced by forms of the imperfect stage Phomopsis occulta on the same media, so far as the amount and type of aerial hyphal growth and the colouration of the aerial stratum and the mid and substrata were concerned. There was this difference observed however. Culture forms derived from ascospores showed a greater tendency to produce large pulvinate, or variously shaped stromata in which perithecial initials eventually formed; accompanied by the imperfect stage; whereas culture forms derived from pycnosporos almost invariably produced only pycnidiospores. Only in one case was the perfect stage obtained in culture, from strains originally obtained from the imperfect stage in nature.

On Sugar-Corn Meal Agar. Growth was at first strongly appressed, colourless, silky; there was a gradual development of a vigorous whitish cottony aerial growth, which as the colony aged became fibrous, somewhat coarsely matted, grayish, in colour, frequently tinged with otter brown; below in the mid and substrata old olive green (olive brown) colour developed which with age became blackish. This darkening was intensified in certain areas, e.g., along the pellicle, where dark fuliginous stromata had formed. Stromata oozed colourless, amber coloured or blackish beads of moisture.

On Leonian's Agar. Growth on this medium was very similar to that on the corn meal medium except that the initial aerial growth was not nearly so vigorous. Within two weeks a dull olivaceous colouring which gradually darkened, was evident in the mid-and substrata. The aerial hyphae also become low, matted, and mottled grayish and whitish. Mature perithecial fruit bodies were not observed to form on this medium.

On Oat Agar. Aerial growth on the hard oat agar medium was exceedingly vigorous. A whitish cottony growth was tinged with otter brown gradually becoming grayish, or olive brown colour. Large stromata formed, and a dark meandering line was frequently observed along the pellicle. This dark line appeared to circumscribe an irregular whitish area of tissue, associated/

associated with a stromatic outgrowth of plectenchymatic tissue. Fertile perithecia of Diaporthe conorum were produced on this medium.

As regards perithecial development in culture the best results were obtained by growing the isolated forms on natural media,—sterilised twigs of conifer and broad-leaved hosts, D. conorum fruited on Pseudotsuga Douglasii, Alnus sitchensis, Acer Pseudoplatanus, and Ulmus campestris. Upon the last named host the amount of perithecial production was astounding both with regard to the number of perithecia produced and the splendid development of the perithecial beaks (Pl. →.

Cultural Proof of Diaporthe conorum as the Perfect Stage of Phomopsis occulta.

The cultural evidence obtained in proving Diaporthe conorum to be the perfect stage of Fuckel's fungus, Phomopsis (Phoma) occulta is presented in the following series of cultural studies of individual forms of the fungus procured mostly from Scotland and from England. It was the writer's purpose at the outset of these experiments to repeat the results previously obtained by WILSON (76, p. 11) on the basis of which he maintained Phomopsis Pseudotsugae to be the imperfect stage of D. conorum (syn: D. pitya). Accordingly, collections of the fungus (at that time regarded by the writer under the specific name D. pitya), were made in localities where Wilson had collected the fungus for his earlier investigations. Diaporthe/

Diaporthe conorum n^o 43931. This specimen originally identified as D. pitya, was collected on a dead suppressed branch of Pseudotsuga Douglasii by Dr. Wilson and the author, at Bowmont Forest, Kelso, Roxburghshire, Scotland, May 1926. Four monoascospore isolations were made May 29, 1926, and the spores planted on sugar-corn meal agar. Within four months all the cultures had reproduced the perfect stage D. conorum, at ordinary room temperature. The imperfect stage was not observed.

On November 10, 1927, eleven monoascospores and three monascus reisolation cultures were made of the above first generation ascospores produced in culture, on Leonian's agar. These cultures were not observed to produce the perfect stage. Stromata formed but these appeared to be sterile.

On November 17, 1927, inoculum from seven monoascospores and three monascus reisolation cultures made on November 10th were used to inoculate sterile twigs of Douglas fir (Pseudotsuga Douglasii). The tubes were placed out of doors in a cold greenhouse in partial sunlight. After three months abundant stromata were plainly visible amongst the matted grayish white hyphal growth covering the twigs. At this time the imperfect stage, Phomopsis occulta was observed in monoascospore culture, 43931 B^{VII} producing three types of spores A, B and intermediate. In twig culture 43931 B^I derived from a single ascus, immature were asci were found. Three months later, May 16, 1928/

1928, the fully matured perfect stage D. conorum was found in every tube, along with the imperfect stage, P. occulta. The erumpent pycnidial fruit bodies varied greatly in size and showed their compound nature by several prominent ostioles emerging from a given fruiting structure.

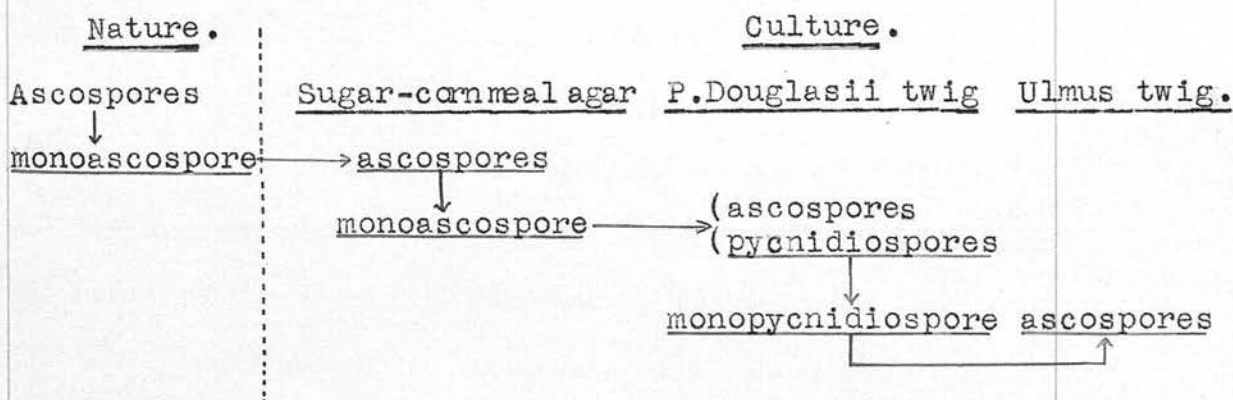
On March 8, 1928, eight monopycnidiospore isolations were made on oat agar from Douglas fir twig culture 43931 B^{VII}. These pycnidiospores had been produced ⁱⁿ monoascospore culture, and were associated in culture with second generation ascospores. In order to test the effect of Taka-diastase, a commercial fungus extract, which McCORMICK (39) had found so successful for the stimulation for the perithecial fruit bodies of *Thielavia basicola*, Zopf in culture, was added to the monopycnidiospore isolation cultures of March 8th, in which by this time growth was well started. The ~~Taka~~ diastase was made up in 1%, 2%, 4% and 6% water solution and filtered through a Berkefeld filter; about 0.5 cc. was added to a given culture. Two tubes each of the isolation set were treated respectively with the different percentage solutions. The perfect stage was not observed to form.

On June 29, 1928, sub-cultures from the eight monopycnidiospore isolations made on oat agar March 8th, were made to sterile twigs of Alnus sitchensis (8) and Ulmus campestris (11). These tubes were kept out of doors in the cold greenhouse. *Perithecia*/

Alnus

The evidence for the connection of Diaporthe
conorum and Phomopsis occulta is given in the following diagram:

Diaporthe conorum.
(*Pseudotsuga Douglasii* form).



D. conorum (Phomopsis occulta) no. 43937. This specimen was obtained from a diseased plantation tree of Abies pectinata, Murthly, Perthshire, Scotland, May, 1926. Cross sections made at the time of pycnidia occurring in the upper layers of the bark of a dead twig revealed the presence of perithecial initials in an entostroma circumscribed by a dark line/

line. Cultures were obtained in this case from the advancing edge of a colony procured from a spore tendril planting on corn meal sugar agar.

On December, 18, 1926, a sterile Douglas fir twig was inoculated with a culture of the fungus and placed out of doors in the cold greenhouse. After six months abundant perithecia with mature spores of D. conorum were observed abundantly throughout the cortex of the twig.

On June 30, 1927, eight monoascospores and four monoascus isolations were made from the perfect stage produced in culture on the Douglas fir twig just referred to. These isolations were grown on corn meal sugar agar. The growth characteristics obtained in this set of cultures were identical throughout, and were in agreement with those observed for D. conorum, no. 43931, from the Douglas fir host, on the same medium. Scattered, large, blackish stromata formed in all the tubes; these oozed abundant droplets of moisture, but they were not observed to contain spores. One particularly large stroma, 4 mm. wide and 3 mm. deep, was removed from the culture tube (43937A^I) and placed on the surface of an agar plate of dilute 3% corn meal agar. Within three weeks both A and B spores of Phomopsis occulta were oozing. In this case the imperfect stage was produced from a monoascospore culture which had been obtained originally from a poly-pynicidiospore culture. The perfect stage was not observed in this culture set.

On/

On November 14, 1927, six tubes of sterile Douglas fir twigs were inoculated with inoculum from the foregoing corn meal cultures, — four monoascospore culture, and two *monoascus* culture inoculations.

Within three months abundant stromata were observed among the matted hyphal growth covering the twigs. Three months later perithecia of *D. conorum* were found in every tube. The imperfect stage was not observed.

D. conorum no. 43940. This specimen from the diseased terminal of a Douglas fir transplant was collected at Glentress, Peeblesshire, Scotland. The lesion had apparently been caused by frost injury. The fungus involved in this lesion was not fruiting at the time of collection. Cultures were made from inner tissue taken at the base of the die-back lesion.

Culture characteristics on sugar corn meal agar were identical with those obtained on the same medium for no. 43931 and no. 43937. thereby indicating that the fungus was very probably *D. conorum*. The same large irregularly, ~~shaped~~ cushion-shaped stromata formed, which oozed droplets, of moisture which were not found to contain spores. A few of these stromata were transplanted to dilute 3% corn meal agar plates, to induce the formation of conidial locules or perithecia by supplying a fresh moisture and a new supply of nutriment which was not over rich. Fruiting of any sort was not obtained.

A sterile Douglas fir twig was inoculated on December 18, 1926, with a culture of 43940 B¹ and placed out of doors in the cold greenhouse in partial light. The perithecia of D. conorum were produced in abundance within six months. The imperfect stage was not discovered.

D. conorum no. 43989. This specimen was collected by Dr. Wilson and Mr. M.E. Mason at Glentress, Peebles-shire, Scotland, September, 1927. The fungus was collected on a dead branch of Pseudotsuga Douglasii. ? E.W.

On October 12, 1927, fourteen monoascospore and one monoascus isolations were made and grown on sugar corn meal agar. The culture characteristics were identical throughout this set of tubes, and agreed with previous isolation cultures of D. conorum on this medium. By March of the following year all the tubes which had been kept outside, part of the time in partial sunlight, and part of the time in the full sunlight, had developed the same large pulvinate stromata; similiar stromata had also formed on this medium in other D. conorum isolation culture sets. These stromata were not observed to produce oozing spores. Several of the compound bodies were crushed in order to discover possible spore locules, but no spores were found.

On November 8, 1927, a parallel set of cultures were made on beet wort agar to test out this medium as regards the stimulation of perithedial production. Culture characteristics on beer wort agar were/

were very much like those on the corn meal sugar agar, with this exception; there was a greater development of the olive brown colour in the aerial stratum, with tinges of otter brown. Large stromatic bodies likewise formed on this medium as one might expect on a medium which so greatly favoured the vegetative growth of the aerial mycelium. In March of the following year an orange-yellow tendril of spores was noted oozing from a large stroma in one of the tubes, from a monoascospore culture. In June the same stromatic body was observed to ooze other tendrils or droplets of spores from multiple ostioles or beaks which now studded this compound fruiting structure. Abundant A spores of Phomopsis occulta occurred in abundance; perithecia of D. conorum were not formed.

On November 14, 1927, another set of cultures using "natural media" of Douglas fir was made. The inoculum was derived from the original isolation culture set on corn meal agar. Twelve monoascospore and one monascus cultures were used in this inoculation set. The tubes were kept outside over winter in the cold greenhouse. Within three months abundant stromata were observed throughout the matted grayish aerial hyphal growth covering the twigs. Within four months after inoculation two monoascospore isolations produced the imperfect stage, Phomopsis occulta was found. These imperfect spores agreed in shape and in size with those produced in nature; the sporophores likewise were of the same shape and size as those/

those found under natural conditions. B spores were not observed. Within six months the mature perfect stage D. conorum had appeared in every tube while only in three of the tubes had the imperfect stage formed.

D. conorum n^o 43979. This specimen on frosted plantation stock of Sitka spruce (Picea Sitchensis) was collected by Mr J.S.L. Waldie, Oxford, England, February, 1928. This specimen was identified by the author as occurring in association with Phomopsis occulta, P. conorum, Dermatea intermedia, Wilson, n. sp. and Myxosporium sp. In isolating the Diaporthe it became indeed interesting to observe which of the two imperfect fungi of the genus Phomopsis occurring on this specimen would be produced in the cultural life-history of the Diaporthe. In both cases the Phomopses were closely associated with the ascomycete although it was noted that P. conorum occurred in the tissues of the upper cortex somewhat removed from the area in which the D. conorum occurred associated with P. occulta, fruiting in the ectostroma.

On April 5, 1928, thirteen monoascospore and two monoaous cultures were made on sugar-cornmeal agar. Culture characteristics were identical throughout this set and showed agreement with previous isolation cultures on this medium. Neither the imperfect nor the perfect stage was obtained.

On/

On May 9, 1928, eleven sub-cultures from the preceding set were made on oat agar, and after colony growth had become well established, sterile Taka-diastase solution (0.5 cc. to each tube), was added as follows:

Cultures 43979 B ^{II, VIII, X, XI, XII}	-1% Taka-diastase sol.
" " B ^{VI, VII,}	-2% " " "
" " B ^{IX, XIII, XIV, XV.}	- <u>untreated.</u>

The mature perfect stage D. conorum was produced in culture 43979 B^{II} monoascospore isolation, within two and one half months. This tube had been treated with 1% Taka-diastase solution. Within six months fertile perithecia had been formed in all the untreated tubes and only in 43979 B^{II, XI, XII} of the Taka-diastase (1%) treated tubes. It would appear that the fungus extract did not appreciably stimulate the production of the perithecial stage. The imperfect stage, Phomopsis occulta was found in all the tubes in association with the perfect stage. P. conorum did not appear in the life-history of the ascomycete.

On April 25, 1928, sterile twigs were inoculated with sub-cultures from the isolation set made April/

April 5, 1928, as follows:- Picea sitchensis, five twigs,- four monoascospore and one monoascus culture; Ulmus campestris, six twigs,- five monoascospore and one monoascus culture; Alnus sitchensis, two twigs,- two monoascus cultures; Pseudotsuga Douglasii, one twig,- one monoascospore culture. The tubes were kept in the cold greenhouse in the full sunlight. One month after inoculation the imperfect stage Phomopsis occulta was observed in nine of the fourteen twigs inoculated and on all of the hosts excepting the Douglas fir. The mature perfect stage Diaporthe conorum was not observed until three months after inoculation when all the elm twigs showed an astounding development^{of}/perithecia with long ostioles (Pl. VI. Fig. 1.) The vigour of the perithecial development on this host exceeded that produced on any other natural host medium. D. conorum also was produced on all of the Alder twigs, on only two of the Sitka spruce twigs (one monospore, and one monoascus culture), and on the Douglas fir twig. D. conorum did not appear in the life-history of this form of Diaporthe.

A parallel Douglas fir twig (Pseudotsuga Douglasii) inoculation set with the one just described was performed, May 3, 1928. This set included inoculations where two monoascospore strains were used instead of one strain. In all five twigs were inoculated with two single line strains each derived from one spore. One twig of Alnus sitchensis was included in this experiment, the purpose of which was to test whether/

whether the presence of two monoascospore strains inoculated together might not have some marked effect in stimulating not only a more abundant production of perithecia, but also perithecia produced in a shorter period of time. As a check on the experiment seven twigs were inoculated with monoascospore strains only. Shortly after a period of three months the mature perfect stage D. conorum was observed in all the tubes save two of the single spore strain check tubes. In the case of the alder twig the imperfect stage, Phomopsis occulta, also formed abundantly. Mature pycnidia of this fungus were only found in one Douglas fir tube that had been inoculated with two single ascospore strains. No difference could be observed in the amount or rapidity of production, of the perfect stage between twigs inoculated with one monoascospore strain, and twigs inoculated with two single spore strains.

Conclusions.

It can now be definitely stated on the basis of the life-history cultural data just submitted, that Diaporthe conorum is the perfect stage of Phomopsis occulta. Whether or not another imperfect fungus also exists in the life-history of this ascomycete is yet to be satisfactorily proven. Two species of imperfect fungi representing two different genera are known to occur in the life-history of a given/

given perithecial form, e.g., Graphium and Cladiosporium in the life-history of Ceratostomella. If Wilson's results in which he stated that D.pitya (= D. conorum) was the perfect stage of P.Pseudotsugae can be finally proven it will be the first case on record where an ascomycete has two imperfect stages both belonging to the same imperfect genus.

Phomopsis conorum did not occur in the life-history of D. conorum.

Thirty-six out of forty monoascospore isolation cultures from forms of Diaporthe conorum on Douglas fir, Abies and spruce reproduced the perfect stage on natural media. In a number of instances the imperfect stage occurred in direct association with the Diaporthe in such cultures.

Ten monoascus isolation cultures also inoculated into sterile twigs (natural media) reproduced the perfect stage. The imperfect stage P.occulta appeared in these cultures.

Perithecia of Diaporthe conorum were observed to form most readily upon sterile twigs in the natural media tests, whereas in those tests where synthetic, oat or sugar-corn meal hard agars were employed, the perfect stage formed infrequently. On such media there was a tendency to form large sterile stromatic bodies in which pycnidial locules or perithecia seldom developed. Taka-diastase, a commercial fungus extract did not give evidence in these experiments/

experiments of stimulating appreciably the formation of the perfect stage on hard agars.

Perithecia formed in the laboratory at ordinary room temperature, or out of doors in a cold greenhouse both in partial sunlight and in the full sunlight. Fertile perithecia formed in two and a half to six months.

The presence of two monoascospore strains inoculated into the same sterile twig did not appear to hasten the formation of perithecia, or otherwise affect the abundance of their production. Monoascospore strain isolations reproduced the perfect stage just as readily as the foregoing.

The perfect stage was obtained from monopycnidiospore isolations on natural media of elm. These isolations of the imperfect stage were obtained from spores produced in culture in association with ascospores, which represented the second generation in culture. Apparently in these pycnidiospores there existed the potentialities for producing the perfect stage, which were lacking in the large number of strains of Phomopsis (p.132) from which the writer failed to obtain the perfect stage, even though these strains were grown on natural media and under conditions similar to those in which positive results were obtained with the imperfect stage of form no.43931, just cited.

The fact that D. conorum fruited as readily on/

on twigs of broad-leaved hosts as upon twigs/^{of}conifers, indicated probable relationships with forms of Diaporthe occurring on the former. Upon elm twigs the amount of perithecia and vigour of the perithecial bodies was most marked. The writer has not investigated Diaporthe forms on elm or other broad-leaved hosts, to determine relationships in nature which may exist between such forms and the species just described on conifers. Furthermore, the fact that the imperfect stage of D. conorum is now known to occur on hosts representing fourteen conifer genera, and is to be found over a wide geographical range, suggests an even wider host relationship. It will be therefore interesting to note how Wehmeyer, in his forthcoming monograph of the genus Diaporthe treats this conifer species.

PHOMOPSIS OCCULTA, Trav. Fuck. Symb. Nachtr. III, 1875,
p. 23; Sacc. Syll. Fung. III, 1884,
150; Flora Ital. Crypt. II, 1906
p. 221.

Syn: Phoma occulta, Sacc. (1884); non Phoma occulta
Desm. (1849).
Phomopsis occulta, (Sacc.) Trav. (1906).
P. Thujae, Died. (1915).
(?) P. Cryptomeriae, Kitajima et Kamei (c?1927)

HISTORY OF THE FUNGUS.

The original description of Phomopsis
occulta, Trav. was given by FÜCKEL (19). In 1875,
he described the imperfect stage, which he had observ-
ed to be associated with the ascomycetous stage,
Diaporthe occulta (Fuck.) Nke., on cone scales of
Picea excelsa in Germany. SACCARDO (56, III, 150)
merely supplied the name and rather unfortunately,
for the specific name occulta was already occupied
by another member of the genus Phoma, -P. occulta,
Desm. (1849) on PHRAGMITES (12). The combination
Phomopsis occulta (Sacc.) made by TRAVERSO (66, p. 221)
in 1906 and listed by DIEDICKE (13, p. 27) must be
altered. It therefore becomes Phomopsis occulta,
Trav.

The present investigation of Phomopsis
occulta has shown that the species, P. Thujae described
by DIEDICKE (15) in 1915, on dead twigs of Thuja
orientalis, L. is only a form of the older fungus.

In/

In 1917, von HÖHNEL (33) had suggested this relationship when he stated that perhaps "P. Thujae, v.H"⁽¹⁾ was only a form of P. occulta, (Sacc.), Trav. or "P. conorum v. H"⁽²⁾. At the same time he recognised the fungus P. occulta as occurring on spruce shoots, as well as upon cones.

Undoubtedly Phomopsis occulta has been frequently confused with P. conorum, a fungus which is now known not to occur in the life-history of D. conorum. It is unfortunate that the latter species should bear the specific name of the perfect stage to which it does not belong.

As a result of the investigation of forms of Phomopsis occulta the species is now known to be widely distributed in Europe and North America on a large number of generic hosts. This investigation was commenced by the author in America (24) when he undertook the identification of a comparatively large number of forms, which appeared to be closely related morphologically to P. juniperovora, a parasite on the Cupressaceae. Further critical studies showed that with/

(1)

Apparently a misquotation for Died.; for Diedicke was the author of this combination.

(2)

Another misquotation (see p. 155).

with one possible exception, (1) the *Phomopsis* forms placed by him in an arbitrarily segregated group, discussed in his earlier paper (24) are now to be considered as forms of *P. occulta*. It would appear therefore that this species is not to be regarded as a fungus new to the United States (64, p. 139) but one widely distributed in that country on a wide variety of hosts.

- (1) The form of *Phomopsis* isolated from *Cephalotaxus drupacea*, Sieb. et Zucc. degenerated in culture, so that further investigation of this form could not be made. The form will be reported later.

EXAMINATION OF EXSICCATI.

The pycnidia and spores of Phomopsis occulta could not be discovered in the type specimen of Diaporthe (Valsa) occulta no. 622, Fungi rhenani (1863) distributed by Fuckel, although ascospores were obtained for study. pycnidia may be found in other exsiccati distributed by Fuckel elsewhere, in which event these must be taken as the type.

Undoubtedly in describing the imperfect fungus which we now know to be definitely associated with Diaporthe conorum, we were dealing with the same fungus which FÜCKEL (19) observed in 1875. At that time he stated "After many years I succeeded in finding the rare pyrenomycete (Diaporthe occulta(Fuck.) Nke.), this time in association with numerous spermagonia which occupied the young stromata to be found on the outer surface of the scales, whilst on the covered scale portion, always below, a circumscribed stroma with perithecia occurred."

The following exsiccati also have been examined:

no.(?) Phoma (Diaporthe) conorum, cone scales of Picea excelsa, coll.Lb.(Herb. Sacc., Padua).

This fungus is Phomopsis occulta.

no.236. Diaporthe occulta, Nke., cone scales, Pinus sylvestris col. Cook, Fung. Brit. ed. secundo (Herb.Kew).

This fungus specimen contains the im-

perfect stage P. occulta.

no.1018. Phomopsis Thujae, Died. Branches of Thuja occidentalis, Syd. Mycoth.germ. (Type spec.)

This fungus is Phomopsis occulta.

DESCRIPTION OF THE FUNGUS.

Phomopsis occulta, Trav., descr. ement.

Pycnidia ectostromatic, scattered or aggregate, simple or compound arising within and seated upon the cells of the upper cortical tissue of the host, amongst whose cells the fruit body is more or less incorporated; partially erumpent; black, carbonaceous, cone-shaped, lenticular, sub-globose, or truncate, with or without a definite ostiole; inner pycnidium of simple fruit body formed from a single primordium,—unilocular, cavity formed in one plane with a thickened layer of pseudo-parenchymatous tissue above; cavity with protrusions from the side walls, hymenium thereby convoluted lined with sporophores; compound pycnidium formed by the fusion of several primordia multi-locular, chambers also tending to form in one plane and fusing to form an elongate, irregular chamber, 0.1 -1.0 x 0.1-0.5 mm. (Pl. XIII, Figs. 1 & 2). Spores of three types A, B and intermediate: A type (Pl. VII, Fig. 1) hyaline, unicellular, generally oblong-elliptical, with obtuse extremities, intermixed with occasional spores, drawn out and pointed at one end, acute or subacute, extreme range (300), 5.0-12.4 x 1.6-3.4 μ , commonly (300), 6.2-9.3 x 1.9-3.1 μ , with two or three oil drops; B type (Pl. VII, Fig. 3) hyaline, unicellular, filamentous, slightly curved or straight, becoming bent at one end like a walking stick, extremities tapering acute or subacute, extreme range (162) 15.2-32.4 x 1 μ , commonly/

commonly (162) 20.9 - 27.0 x 1 μ , with several small guttules; intermediate type (Pl. VII, Fig. 2) frequently occurring intergrading between the A and B types, very irregular in shape, attenuated, approaching the filamentous type, extreme range (80) 8.7-15.5 x 1.2-2.5 μ , commonly (80) 9.3-13.3 x 1.2-2.2 μ ; sporophores (Pl. VII, Fig. 4) flexuous, subulate, with tapering acute or subacute extremities, persistent. Both A and B type spores produced on the same type of sporophore; extreme range 5.6-15.8 x 1.0 - 2.8 μ ; spores exuded in a whitish or yellowish tendrill.

The imperfect stage of Diaporthe conorum

(Desm.) Niessl.

Hab. Widely distributed throughout Europe on the fallen cones and dead stems of Picea excelsa, Cupressus sempervirens, Pinus sylvestris, Thuja sp., Abies pectinata, A. grandis and Pseudotsuga Douglasii (see Diaporthe conorum, p. 59). According to Diedicke on the dead branches of Thuja occidentalis (Syn: Phomopsis Thujae) in Germany.

The author has collected or identified the fungus on dead parts of branches or trunks, of fourteen coniferous host genera in North America and in Europe as follows: -

United States

- Taxus baccata, L., T. baccata, var. fastigata, Loud., T. cuspidata, Sieb. et Zucc., Taxodium distichum, (L.) Rich., Sequoia sempervirens (Lamb.) Endl., S. gigantea, D.C., Cryptomeria japonica, Don., Larix/

Larix leptolepis, Murr.; L.europaea, D.C., Pseudo-
tsuga Douglasii, Carr., P. glauca, Mayr., Tsuga
Canadensis (L.) Carr., Abies homolepis, Sieb. et
 Zucc., A. Veitchii, Murr., Picea pungens, Engelm.,
Juniperus virginiana, L., Cupressus sempervirens, L.,
Thujaopsis dolobrata, Sieb. et Zucc.; Canada(British
Columbia), P. Douglasii; Great Britain,— P.Douglasii,
A. pectinata, D.C., Picea Sitchensis, Carr., P.excelsa,
 Link., T. plicata, Don., Pinus sylvestris, L., Larix
europaea; Denmark,— P. Douglasii; Holland,—
P. Douglasii; France,— A. pectinata.

In Scotland the author has frequently
 found the fungus associated with frost damage amongst
 nursery or transplant stock of P. Douglasii.

SPORE SIZE OF PHOMOPSIS OCCULTA.

Variation of A. Spore Size in Nature.

The size of the A spores of Phomopsis occulta was first studied on the Douglas fir host. Three hundred spores of the oblong elliptic type, the shape recognised as typical for the species, were measured from 10 collections of Douglas fir forms secured from the United States, Great Britain and Denmark. These measurements were treated biometrically.

A further study of forms of the species on 13 generic hosts other than Douglas fir, showed a very close agreement between the size of spores produced on the various hosts, and those produced on Douglas fir. In TABLE XIII., it will be observed that only a slight variation in size occurred amongst the various host forms, and that this variation was within the range determined for the Douglas fir forms. The forms presented in this table were collected from both North America and Europe. With regard to variability the forms which seemed to fluctuate most were those collected on the Sequoia gigantea and Thujaopsis dolobrata. In these particular instances only a scant number/

number of abnormal A spores, were produced along with an abundance of B spores. The length and width of 380 spores of forms on 13 host genera showed a size average and range practically coincident with that of 320 spores of forms on Douglas fir. Saccardo gave the measurements of Phoma occulta as being 7 x 3 μ .

<u>Cryptomeria japonica</u> *	10	7.1 x 2.8	5.6- 8.7 x 2.2-3.1
<u>Pinus sylvestris</u>	10	7.6 x 2.3	6.5- 8.1 x 2.2-2.5
<u>Juniperus virginiana</u> *	20	7.6 x 2.5	6.5- 8.7 x 2.2-2.8
<u>Cupressus sempervirens</u> *	10	7.9 x 2.4	6.9- 8.7 x 2.2-2.5
<u>Thuja plicata</u>	50	7.2 x 2.2	5.3- 8.7 x 1.6-2.8
<u>Thujaopsis dolobrata</u> *	10	6.6 x 2.6	5.6- 7.1 x 2.2-3.1
<hr/>			
Total - 13 host genera	380	7.5 x 2.4	5.0-12.4 x 1.6-3.4

* Forms collected in the United States; forms on Larix and Pseudotsuga collected on both sides of the Atlantic have been grouped for convenience.

(TABLE XIII) AVERAGES and EXTREME RANGES of A. SPORES, P. OCCULTA, on DOUGLAS FIR and other HOSTS COMPARED.

HOST	NO. SPORES	AVERAGE LENGTH X WIDTH, μ .	EXTREME RANGE LENGTH X WIDTH, μ .
<u>Pseudotsuga Douglasii</u>	320	7.4 x 2.4	5.3-12.4 x 1.6-3.1
<u>Abies pectinata</u>	50	7.9 x 2.4	6.2-12.4 x 1.9-3.4
<u>A.homolepis</u> *	10	7.7 x 2.4	6.8-10.5 x 1.9-2.5
<u>A.Vietchii</u> *	10	7.2 x 2.2	5.3-8.1 x 1.9-2.5
<u>Picea excelsa</u>	10	7.8 x 2.1	5.6-9.0 x 1.9-2.5
<u>Larix Europaea</u>	80	7.5 x 2.4	5.6-10.2 x 1.9-3.1
<u>Tsuga canadensis</u> *	10	7.8 x 2.5	6.2-9.9 x 1.9-3.1
<u>Taxua baocata</u> *	10	7.9 x 2.4	6.2-9.3 x 2.2-2.8
<u>T. baocata fastigiata</u>	20	7.5 x 2.4	5.9-11.2 x 1.9-3.1
<u>T.cuspidata</u> *	10	7.6 x 2.3	6.2-9.3 x 1.6-2.8
<u>Sequoia semper virens</u> *	40	7.7 x 2.3	6.2-10.2 x 1.9-3.1
<u>S.gigantea</u> *	10	5.6 x 2.0	5.0-6.8 x 1.6-2.2
<u>Taxodium distichum</u> *	10	7.4 x 2.4	5.6-8.1 x 2.2-2.8

PHOMOPSIS THUJAE DIED. A FORM OF P. OCCULTA.

The biometrical constants derived from measurements of spores from specimen No.1018, Phomopsis Thujae ~~Died~~ Sydow, *Mycotheca germanica* were compared with the species P.occulta on Douglas fir. A significant difference was not obtained, as is shown in TABLE XIV. The spore shape in each case was identical. A form of P.occulta (No.43939) isolated from Thuja plicata showed spore size and shape agreement with Diedicke's fungus; culturally it agreed with forms of P.occulta from other hosts.

TABLE/

(TABLE XIV) BIOMETRIC CONSTANTS of P. THUJAE COMPARED with the
P. OCCULTA

POPULATION	NO: SPORES	M μ	LENGTH		STANDARD DEVIATION	COEFFICIENT of VARIABILITY
			SIGNIF. of MEAN DIFFERENCES SIGNIF. from			
<u>P. occulta</u>	300	7.5 ±.040			1.016 ±.028	13.54 ±.380
<u>P. Thujae</u> (type)	100	7.2 ±.052	none		.776 ±.037	10.77 ±.520
WIDTH						
<u>P. occulta</u>	300	2.4 ±.015			.380 ±.011	15.83 ±.447
<u>P. Thujae</u> (type)	100	2.3 ±.022	none		.332 ±.016	14.42 ±.702

PHOMOPSIS CRYPTOMERIAE, A FORM OF P. OCCULTA.

Phomopsis Cryptomeriae. Kitajima et Kamai (35), recently described in Japan, and attributed as the cause of a 'branchwither' disease of Cryptomeria japonica, possibly may be regarded also as a form of P.occulta. The measurement ranges given by the authors of the fungus are very suggestive, ^{spores} A-/6.4-8.1x2.1-2.5 μ , and for the B spores, 28.0-33.6x8-1.0 μ . The range is close to that given by the authors for a form of P.occulta on Cryptomeria japonica in the United States (see TABLE XIII). The A spores of the Japanese form are described as being oblong in shape with two guttules. However until the writer is able to study this form in culture he is not in a position to say definitely that the organism on Cryptomeria in Japan is not a new species.

Variation of A Spore Size in Culture.

The size of the A spores produced in culture agreed surprisingly with those examined in nature. Variation occurred, but within the specific range determined for the fungus in nature. In TABLE XV. the very close agreement which was found between spores produced in culture and nature, by Douglas fir forms of the species P.occulta, is shown. The spores in culture were produced on sugar-corn meal, Leonian's and oat agars, and on the following natural media, - twigs of Pseudotsuga Douglasii, Acer, Ulmus and Alnus. While it is not possible to give here, in detail, the data secured by the measurement of spores of P.occulta, from individual forms in 45 cultures, it can be said that in certain instances the averages and ranges of culture and nature produced spores were practically coincident.

Apparently the artificial substratum had little or no effect in influencing the size of the spores. The spore size of culture produced spores varied slightly, but as in nature, within the specific range. TABLE XVI. allows for a comparison of the average size and range of spores in the various media studied. It will be noted that on elm the spores tended to be small; elm was ordinarily regarded as a very congenial host substratum for Diaporthe conorum (P.occulta.)

(TABLE XV) SIZE AVERAGES and RANGES of SPORES of P. OCCULTA (DOUGLAS FIR FORMS) PRODUCED in NATURE and CULTURE COMPARED

POPULATION	NO. SPORES	AVERAGE LENGTH X WIDTH, μ .	AVERAGE RANGE LENGTH X WIDTH, μ .	EXTREME RANGE LENGTH X WIDTH, μ .
<u>P. occulta</u> (11 forms) Nature	320	7.4 x 2.4	6.4-7.9 x 2.1-2.6	5.3-12.4 x 1.6-3.1
<u>P. occulta</u> (7 forms) Culture	480	7.2 x 2.4	6.1-7.8 x 2.0-2.6	5.0-9.3 x 1.6-3.1

(TABLE XVI) AVERAGES and RANGES of SPORES of *P. OCCULTA* PRODUCED on ARTIFICIAL
HARD AGARS and NATURAL MEDIA, COMPARED

SUBSTRATUM	NO. SPORES	AVERAGE LENGTH X WIDTH, μ.	AVERAGE RANGE LENGTH X WIDTH, μ.	EXTREME RANGE LENGTH X WIDTH, μ.
<u>Pseudotsuga</u> <u>Douglasii</u> (Nat.)	320	7.4 x 2.4	6.4-7.9 x 2.1-2.6	5.3-12.4 x 1.6-3.1
Sugar-corn meal agar	160	7.2 x 2.3	6.8-7.5 x 2.0-2.5	5.6-9.3 x 1.6 -3.1
Oat agar	80	7.1 x 2.3	6.9-7.2 x 2.3 -2.5	5.6-9.0 x 1.6-2.8
Leonian's agar	70	7.1 x 2.4	7.0-7.3 x 2.3-2.5	5.0-8.7 x 1.9-3.1
<u>P. Douglasii</u> twig	90	7.5 x 2.3	7.2-7.8 x 2.2-2.5	6.2-9.3 x 1.6-2.8
<u>Acer Pseudoplatanus</u> twig	40	7.4 x 2.5	6.9-7.9 x 2.4-2.6	6.2-9.3 x 1.9-3.1
<u>Ulmus campestris</u> twig	30	6.7 x 2.3	6.1-7.0 x 2.2-2.3	5.9-8.1 x 1.6-3.1
<u>Alnus Sitchensis</u> twig	10	7.3 x 2.3		6.2-9.3 x 1.9-2.5

In order to estimate further any influence the artificial substratum might have in affecting spore size, measurements were made of spores of forms from seven generic hosts, produced on both hard agars and natural media, - the same media used for the Douglas fir forms presented in TABLE XVI. These comparisons between naturally and culturally produced spores are presented in TABLE XVII. The substratum seemed to have little or no influence in affecting spore size. Slight variation occurred, but within the given specific range. Spores of the Thujopsis form regained their normal size. In nature, these spores as already stated, were produced in connection with abundant B spores and were abnormally small.

(TABLE XVII) AVERAGES and RANGES of SPORES of P.OCCULTA PRODUCED in NATURE
And CULTURE, COMPARED

GENERIC HOST	NATURE or CULTURE	NO. SPORES	AVERAGE LENGTH X WIDTH, μ .	EXTREME RANGE LENGTH X WIDTH, μ .
<u>Ables</u>	Nat. Cult.(9)	70 140	7.8 x 2.4 7.3 x 2.3	5.3-12.4 x 1.9-3.4 5.9-9.6 x 1.6-2.8
<u>Larix</u>	Nat. Cult.(5)	80 50	7.5 x 2.4 7.5 x 2.4	5.6-10.2 x 1.9-3.1 5.3- 9.0 x 1.9-3.1
<u>Taxus</u>	Nat. Cult.(9)	40 90	7.6 x 2.4 7.4 x 2.2	5.9-11.2 x 1.6-3.1 6.2- 9.3 x 1.6-3.1
<u>Sequoia</u>	Nat. Cult.(3)	40 30	7.8 x 2.3 7.9 x 2.3	6.2-10.2 x 1.9-3.1 5.6-10.2 x 1.9-3.1
<u>Thujaopsis</u>	Nat. Cult.(7)	10 70	6.6 x 2.6 7.6 x 2.3	5.6-7.1 x 2.2-3.1 5.9-9.9 x 1.9-3.1
<u>Juniperus</u>	Nat. Cult.(1)	20 10	7.6 x 2.5 7.8 x 2.5	6.5-8.7 x 2.2-2.8 7.1-8.4 x 2.2-2.8
<u>Thuja</u>	Nat. Cult.(4)	50 40	7.1 x 2.2 7.1 x 2.3	5.3-8.7 x 1.6-2.8 5.9-8.7 x 1.9-3.1

Variation of B Spore Size in Nature and Culture.

It was found difficult to measure the B type spores satisfactorily because of their curvature and the crooked ends, hence the measurements given in the following tables only approximate the actual length of the spores. It was the custom in measuring B spores to measure those which were inclined to be straight. Spore width which is approximately 1μ , was not considered.

Despite the difficulty in measuring the B spores the writer found the measurements of a considerable number of the filamentous bodies produced in culture on hard agars and natural media, agreed surprisingly in size with the spores produced in nature. A comparison of naturally and culturally produced B spores is given in TABLE XVIII.

(TABLE XVIII) AVERAGES and RANGES of B. SPORES of P. OCCULTA in NATURE and CULTURE, COMPARED.

NATURE or CULTURE	NO. SPORES	AVERAGE LENGTH, μ.	AVERAGE RANGE LENGTH, μ.	EXTREME RANGE LENGTH, μ.
Nature (9 generic hosts)	162	23.1	20.9 - 27.0	15.2 - 34.4
Culture (44)	442	22.7	20.9 - 26.7	10.2 - 34.1

Variation of Intermediate Spore Size in Nature
and Culture.

A limited number of measurements were made of the intermediate spores, the variously shaped type intergrading between the A and B. Even the intermediate spores evidenced a certain constancy with regard to size, and while variation occurred it took place within a limited range. In TABLE XIX spores in nature, and in culture on hard agars and natural media are compared:

TABLE/

(TABLE XIX) AVERAGES and RANGES of INTERMEDIATE SPORES of P. OCCULTA in
NATURE and CULTURE, COMPARED.

NATURE or CULTURE	NO. SPORES	AVERAGE LENGTH X WIDTH, <i>μ</i>	AVERAGE RANGE LENGTH X WIDTH, <i>μ</i>	EXTREME RANGE LENGTH X WIDTH, <i>μ</i>
Nature (6 generic hosts)	80	10.9 x 1.7	10.0-14.5 x 1.2-2.0	8.7-15.5 x 1.2-2.5
Culture (9)	79	12.2 x 1.5	10.9-14.0 x 1.2-2.0	8.4-17.7 x 0.9-2.5

SPORE SHAPE IN NATURE AND IN CULTURE.

As in the case of its perfect stage, the shape of pycnidiospores, both A and B, of Phomopsis occulta examined over a wide range of coniferous hosts, and from many geographical sources, showed marked agreement with regard to this morphological character.

The oblong elliptic spore with obtuse extremities plainly predominated in all the collections studied. This type of spore was associated frequently in small numbers with an elliptic type which had become attenuated at one end. The occurrence of this second type of spore is indeed characteristic. The shape of B and intermediate spores were in general agreement throughout the host range. What has been stated for the shape of spores in nature, also applied to spores produced in culture. Artificially produced spores did not deviate in shape.

CULTURAL LIFE HISTORY of PHOMOPSIS OCCULTA

In the preceding section Diaporthe conorum has been shown to be the perfect stage of Phomopsis occulta. The fact that the ascogenous form could be obtained from monopycnidiospore isolation cultures from spores formed under artificial conditions, has also been demonstrated. It was the general experience that D. conorum(P. occulta) was readily produced in culture by monospore isolation strains of the fungus.

Throughout the study of the imperfect stage, Phomopsis occulta, 83 forms of this species, representing a wide host and geographical range, have been under observation. These forms have been isolated from single spores, spore dilution colonies, spore tendrill plantings, and from inner bark tissue. In only two cases(one from inner bark tissue, no.43940; one from spore horn planting, no.43937), has the perfect stage been discovered amongst these, although the forms have been grown on media both natural and artificial, which are known to favour the production of the perfect stage, D. conorum. The imperfect stage seems to be thoroughly capable of continually propagating itself, spore generation after spore generation, without the formation of the ascomycetous stage.

As a general rule, forms of Phomopsis occulta freshly isolated from pycnidiospores produced/

produced the imperfect stage vigorously in culture. When cultures appeared to be degenerating as the result of continuous growth on artificial hard agars, fruiting ceased. Such decrease in the production of fruiting bodies in certain instances was associated with the production of B spores only. If A spores occurred these were abnormally small. P. occulta was isolated from the inner cortical tissues of a die-back of Abies pectinata (Jura, France) which occurred on the terminal of a branch which had been girdled by Phomopsis abietina Wilson et Hahn. This isolation, although agreeing with other forms of P. occulta in culture growth characteristics has never produced the A type spore in culture. Only B spores have formed which agree with those regarded as being typical for the species.

The three types of spores - A, B, and intermediate - were, as a rule, readily found both in nature and in culture. An abnormally long A spore also occurred infrequently in nature, and in some of the forms with increased number in culture. In the measurement study of the A spores these spores, while included in the extreme range, were measured only in accordance with the ratio of their occurrence among the normal A spores.

In a limited number of tests, the author /

author was unable to secure the germination of the B type spores. These tests included dilute spore suspensions in sterile distilled water, in 0.5 per cent. malt extract solution, and upon the surface of sugar-corn meal in a Petri dish. In all these media the A spores germinated at room temperature without any difficulty. In no case were the B spores successfully germinated. In one test however slender processes (Pl. VIII, Figs. 1 & 2) were obtained, which resembled aborted germ tubes. The experiment in which these were obtained is described:

June 8, 1927. B spore suspensions in hanging drops of sterile malt extract in fusion, 0.5% were put up and kept at room temperature. Spores were secured from Phomopsis occulta culture, 43965 B⁷, a form obtained from the inner cortical tissue of Abies pectinata France. This culture had only produced B spores.

June 9th. Within 24 hours the B spores produced slender processes usually at that part of the spore where a bend occurred. In diameter these processes were approximately the diameter of the spore itself. Generally the processes were just slightly pushed out; the longest measured was 7 μ long. This particular process was not observed to increase in length nor was there any appreciable increase in length observed in other spores with shorter processes.

CAYLEY (9) has described a similar phenomenon in the case of the B spores of the Phomopsis stage of Diaporthe pernicioso. This has been regarded by some authors as true germination. Cayley did not regard such as being the case. The writer is inclined to take the same view.

Presence of Physiological Forms Within the Species.

A large number of forms of Phomopsis occulta were isolated for culture study in North America and in Europe, from species of conifers representing twelve genera, namely - Taxus, Taxodium, Sequoia, Pseudotsuga, Larix, Picea, Abies, Pinus, Juniperus, Thuja, Thujopsis. Forms obtained from different species among these genera showed agreement with each other and with forms of different genera. Certain slight differences were observed to occur culturally among the forms, e.g., in the tendency to produce abundant pycnidia or a lesser number of fruit bodies, in the intensity of colour production, in the amount of aerial hyphae produced. Such differences could only be interpreted as indicating physiological forms within the species. Fundamentally throughout the group of forms the agreement of growth characteristics was most apparent. This similarity of characteristics is brought out in (Pl. XXI; Pl. XXII).

Culture Growth Characteristics.

Growth characteristics reported for Diaporthe conorum isolations showed great similarity with cultures obtained from pycnidiospores of Phomopsis occulta.

On Sugar-corn meal agar. Colony growth at first appressed, silky, colour colourless, soon covered by a cottony aerial growth which was indistinctly zonate; the aerial hyphae gradually became coarsely matted, mottled, grayish and whitish. Below in the mid-and substrata a dull olivaceous colour developed. Fruiting was usually abundant, the simple or compound stromata occurring over the surface of the colony, exuding spore tendrils or spore masses according to the moisture conditions in the tube. Along the pellicle/

pellicle meandering dark lines were observed delimiting hyphal areas. Otter brown colour may or may not appear, tinging the aerial hyphae.

On Leonian's Agar. Colony growth at first appressed, silky later covered by an aerial growth not so well developed as on corn meal sugar agar. This aerial growth likewise became coarsely matted, and darkish gray or whitish. In the mid-and substrata dull olivaceous colour developed. Fruiting was not nearly so vigorous as upon the maize medium. Scattered, large compound, pulvinate stromata frequently formed.

2. PHOMOPSIS JUNIPEROVORA, Hahn. Phytopath. X, 1920, p.249.HISTORY OF THE FUNGUS.

Phomopsis juniperovora, Hahn, which is a well known parasite of nursery seedling and transplant stock in the United States, was described by the author in 1920 (23). In an earlier investigation (22) of the disease artificial inoculation experiments were carried out which proved the parasitic nature of this fungus, at that time provisionally regarded as a species of Phoma. The fungus is now known to be widely distributed in the United States. Up to the present time it is not known to occur in Europe, although an intensive search may reveal its presence on the continent in nurseries where ornamental stock of the Cupressaceae are grown. The writer has searched for the organism in Great Britain, but its discovery up to the present time, has not been made.

In 1919, BOTTOMLEY (3) reported a preliminary investigation into a disease attacking young Cupressus plants in South Africa. The symptoms of the disease were identical with those described for Phomopsis juniperovora, in the United States.

Bottomley called attention to the close resemblance between ^{the} South African organism and the North American one. Several morphological differences were, however, noted. Bottomley did not describe B or intermediate spores/

spores, and the culture growth characteristics also appeared to be distinct. The South African fungus may possibly be regarded as a physiological form of P. juniperovora, and there is also the possibility of its being a distinct species. The former consideration seems to be the more likely.

In 1926, HAHN (24) called attention to forms of Phomopsis which were closely related to Phomopsis juniperovora, on a comparatively large number of genera and species of conifers. The present investigation has shown that these forms so widely distributed so far as their host relationships are concerned, are now to be regarded for the most part as P. occulta (see p. 113). P. juniperovora would appear to be more or less confined to the Cupressaceae (24) so far as the host relationships of this particular Phomopsis are concerned.

DESCRIPTION/

DESCRIPTION OF THE FUNGUS.

Phomopsis juniperovora, Hahn, descr. ement.

Pycnidia ectostromatic without a circumscribing dark line in the cortex, scattered or aggregate, simple or compound; arising within, and seated upon the cells of the upper cortical tissues of the host, amongst whose cells the fruit body is more or less incorporated; partially erumpent; black, carbonaceous, cone shaped, lenticular, sub-globose, or truncate, with or without a definite ostiole; inner pycnidium of a simple fruit body formed from a single primordium, unilocular,, cavity formed in one plane with a thickened layer or pseudoparenchymatous tissue above; cavity frequently with protrusions from the side walls, hymenium convoluted giving rise to sporophores; compound pycnidium formed by the fusion of several primordia, multilocular, chambers tending to form in one plane and fusing to form an elongate, irregular chamber, 0.1-0.6 mm. diam. Spores of three types A, B and intermediate; A type, hyaline, unicellular, typically elipsoid with subacute extremities, and with a "pinching in" or narrowing at the median part of the spore, asymmetrical or symmetrical, also occasionally oblong-elliptic with obtuse extremities, or elliptic fusoid (spindle shaped), extreme range (100), 5.6-11.5 x 1.9-3.1 μ commonly (100), 7.5-10.0 x 2.2-2.8 μ , biguttulate (Pl. IX, Fig. 1); B type, hyaline, unicellular, /

unicellular, filamentous, slightly curved, flexuous, tending to become straight, not pronouncedly hooked or bent at one end, as in P. occulta, extremities tapering, one extremity frequently obtuse, extreme range, 17.1 -34.0 x 1 μ , commonly 20.2-26.9 x 1 μ , with several small guttules (Pl.IX, Fig.3); intermediate type, occurring infrequently, intergrading between the A and B types, very irregular in shape, attenuated, approaching the filamentous type, extreme range, (20), 9.9-17.7 x 1.6-2.5 μ . (Pl.IX, Fig.2); sporophores, subulate, persistent, both A and B type spores produced on the same type of sporophore, extreme range, 5.9-25.7 x 1.0 - 3.1 μ , commonly 11.2-14.3 x 1.0-2.5 μ , (Pl.IX, Fig.4); spores exuded in a whitish or yellowish tendril.

Perfect stage unknown.

Hab. Widely distributed in the United States in nurseries and in ornamental plantings where species of the Cupressaceae are grown. Hosts, — Juniperus virginiana, L., J. scopulorum, Sarg., J. lucayana, Britt., J. pachyphloea, Torr., J. communis, L., J. communis var. Montana, Ait. J. horizontalis, Moench (J. prostrata, Pers.) J. procumbens, Sieb. (J. chinensis var. procumbens, Endl.) J. chinensis var. Pfitzeriana, Spaett, J. sabina, L., J. sabina var. tamariscifolia, Ait.; Cupressus sempervirens, L. C. macrocarpa, Hartw., C. Arizonica, Greene, C. goveniana, Gord., C. Benthami, Carr., C. funebris, Endl., C. glabra, Sudw.; Thuja occidentalis, L./

L., T. orientalis, L., T. plicata, Don.,; Chamaecyparis
 (Retinospora) obtusa, Sieb. et Zucc., C. pisifera,
 Sieb. et Zucc., C. pisifera var. plumosa, Beiss., C.
Lawsoniana, Parl. (Cupressus Lawsoniana, Murr.),⁽¹⁾

Bottomley reported a Phomopsis form resembling P. juniperovora on seedlings of Cupressus,— C.
Arizonica, C. macrocarpa, C. torulosa, Don. in South
 Africa.

(1).

Under conditions of artificial inoculation
 HAHN (24) obtained positive results with
Phomopsis juniperovora on Pseudotsuga
Douglasii and Larix europaea; negative
 results were obtained, on Pinus (6 species)
 and Picea (3 species).

SPORE SIZE OF PHOMOPSIS JUNIPEROVORA.Variation in Size of A Spores in Nature.

A measurement study of 100 A spores of Phomopsis juniperovora representing nine forms on Juniperus virginiana collected from widely distributed sources in the United States showed a series of averages which were in very close agreement with each other. Variation in size occurred, but this variation was within a more or less proscribed range. Thirty spore measurements of strains of the fungus on Cupressus gave an average which was practical identical with that from Juniperus. The extreme range on Cupressus was within that determined for the fungus on Juniper. A comparison of these averages and ranges is given in Table XX.

TABLE XX.

AVERAGES AND EXTREME RANGES OF A SPORES OF *P. JUNIPEROVORA* (12 FORMS)
ON JUNIPERUS AND CUPRESSUS, COMPARED.

	Strain No.	No. Spores.	Average length x width, μ	Extreme Range length x width, μ
<i>Juniperus virginiana</i>	25005	20	7.9 x 2.3	6.9-8.1 x 1.9-2.5
"	32965	10	9.0 x 2.5	7.1-11.5 x 2.2-3.1
"	38275	10	8.5 x 2.7	7.4- 9.9 x 2.5-3.1
"	41041	10	8.6 x 2.5	5.6-10.2 x 2.2-2.8
"	41380	10	8.6 x 2.5	7.8- 9.3 x 2.2-2.8
"	41381	10	8.6 x 2.4	7.1- 9.9 x 1.9-2.8
"	41388	10	8.5 x 2.4	6.5- 9.6 x 2.2-2.8
"	41963	10	8.6 x 2.2	7.5- 9.6 x 1.9-2.5
"	43968	10	8.6 x 2.5	6.8-10.9 x 1.9-3.1
Total	(9 coll.)	100	8.5 x 2.4	5.6-11.5 x 1.9-3.1
<i>Cupressus Arizona</i>	38276	10	8.5 x 2.4	6.8- 9.9 x 1.9-2.8
"	41048	10	8.3 x 2.5	6.8- 9.3 x 2.2-2.8
"	41374	10	8.6 x 2.3	7.1- 9.9 x 1.9-2.8
Total	(3 coll.)	30	8.4 x 2.4	6.8- 9.9 x 1.9-2.8

VARIATION OF A SPORE SIZE IN CULTURE.

It was observed that certain of the forms of Phomopsis juniperovora which had been in culture for an extended period of time, lost their ability to produce fertile pycnidia, which appeared only sparingly when the forms were first isolated.

Forms, nos. 41041, 41048, and 41376 isolated from Juniperus virginiana, Cupressus Arizonica and Chamaecyparis obtusa did not lose their ability to produce spores although they had been kept growing in culture for four years. Fruiting was not abundant; the reduced number of spores which formed under artificial conditions were however normal, agreeing closely in size with those produced by form no. 43968 recently isolated from diseased material of Juniperus virginiana.

A measurement study was made of 100 spores produced under artificial conditions on oat and sugar-corn meal agars, and on natural media, - twigs of Douglas fir, elm, Acer and alder. These spores are produced by forms of Phomopsis juniperovora isolated from, Juniperus, Cupressus and Chamaecyparis. It was found that there was a marked similarity in size between the spores produced in culture and those produced in nature. The individual averages for each of the ten cultures studied are not given here but the total average, average range and average size are to be found in Table XXI.

TABLE XXI.

Averages, average ranges and extreme ranges of A spores of P. juniperovora produced in nature and culture compared.

Nature or Culture.	No. Spores.	Average length x width	Average range length x width	Extreme range, length x width
Nature	100	8.5 x 2.4	7.9-9.0 x 2.2-2.7	5.6-11.5 x 1.9-3.1
Culture	100	8.3 x 2.5	7.9-8.9 x 2.3-2.6	6.2-10.2 x 1.9-3.1

As was the case in Phomopsis occulta, artificial growth on hard agars or natural media, did not perceptibly affect the size of spores of P. juniperovora. The size range in culture agreed with that in nature.

B Spores in Nature and Culture.

In nature the B spores of Phomopsis juniperovora were observed occurring commonly. The ratio of A spores to B spores was, however, extremely variable. In the limited number of cultures which produced spores in this present investigation, the B type were found occurring only scantily amongst the A type. Only a very few were obtained for measurement; the extreme range of these was coincident with that of B spores produced in nature.

COMPARISON/

COMPARISON of PHOMOPSIS JUNIPEROVORA
and P. OCCULTA..

At first glance Phomopsis juniperovora and P.occulta resembled each other very closely. It was not until they had been studied culturally, and the minute differences separating the species detected, that they could be distinguished, for both species were found on members of the Cupressaceae upon which the former fungus is known to be parasitic. P.juniperovora on the other hand, is now known only to occur on this host group.

The spore size ranges of the two species closely overlap, in fact so closely, that to separate them satisfactorily the measurements must be treated biometrically. Such a comparison of biometrical constants is given in TABLE XXII. where spore populations of the species Phomopsis juniperovora on Juniper, (10 forms) are compared with the species P.occulta on Douglas fir (10 forms) and with the same species on Thuja (1 form). A significant difference was obtained only in regard to spore length.

In/

(TABLE XXII) BIOMETRIC CONSTANTS of A. SPORES of P. JUNIPEROVORA
 COMPARED with P. OCCULTA (2 GENERIC HOST FORMS.)

POPULATION	NO. SPORES	M	SIGNIF. of MEAN DIFFERENCES SIGNIF. from	STANDARD DEVIATION	COEFFICIENT of VARIABILITY
<u>Phomopsis</u> <u>juniperovora</u>	100	8.7 ±.066		.984 ±.047	11.31 ±.547
<u>P.occulta</u> (1)	300	7.5 ±.040	Signif. from	1.016 ±.028	13.54 ±.380
<u>P.occulta</u> (2)	100	7.2 ±.052	Signif. from	.776 ±.037	10.77 ±.520
<u>Phomopsis</u> <u>juniperovora</u>	100	2.5 ±.025		.364 ±.017	14.56 ±.709
<u>P.occulta</u>	300	2.4 ±.015	none	.380 ±.011	15.83 ±.447
<u>P.occulta</u>	100	2.3 ±.022	none	.332 ±.016	14.42 ±.702

(1) Douglas fir forms; (2) Thuja form.

In further distinguishing the two species other morphological differences were found in the shape of the A spores and their extremities, and in the shape of the intermediate and the B spores. Physiologically they were readily distinguishable in culture.

SHAPE./

SHAPE OF A AND B SPORES IN NATURE AND CULTURE.

One of the distinguishing morphological characteristics which set P. juniperovora apart from P. occulta, was the tendency for the former species to produce A spores which were inclined to be narrowed or pinched in, at or near the middle part of the spore body, so that they appeared almost slipper-like. Spores which were dorsi-ventral and slightly curved became pronouncedly slipper shaped. The extremities of these spores were subacute for the most part, and not generally obtuse as in the case of the A spores of P. occulta. In culture elliptic shaped spores with subacute extremities predominated; the tendency to become pinched in or narrowed at the middle was only slightly expressed.

The B spores in both nature and in culture were flexuous, tending to become straight. They were never observed to be curved, horseshoe shaped, as in the case of P. conorum (Pl.X, Fig.2), or hooked, as in the case of P. occulta (Pl.VII, Fig. 3).

CULTURAL LIFE-HISTORY OF P. JUNIPEROVORA.

The perfect stage of Phomopsis juniperovora has not yet been discovered. Attempts were made to induce the formation of an ascomycetous form by growing six forms of the species on twigs of Pseudotsuga Douglasii, Chamaecyparis Lawsoniana, Thuja plicata, Acer, Ulmus and Alnus, the cultures being kept at approximately freezing temperature for three months, and then brought back to room temperature where they were kept under moist conditions. The perfect stage did not form.

Culture Growth Characteristics.

When the culture characteristics of Phomopsis juniperovora were first reported (23) an empire yellow colour, which became orange rufous with age, was described on corn meal agar. This colour which diffused throughout the medium in advance of colony growth extension, was accompanied by the formation of flaming orange crystals, which formed quickly and abundantly. The same vivid orange-red colouration together with the flaming orange crystals was produced on sugar-corn meal agar.

It was noted that in cultures which became abnormal that this typical colour just discussed, diminished in intensity and ultimately disappeared almost completely. On the contrary cultures which had been maintained in a normal condition of growth, were observed to be capable of reproducing the characteristic/

characteristic colour and crystals after a considerable period of time in culture. Certain of the forms of P. juniperovora which have shown this ability have been in culture four years. It was noticed that on sterile twigs inoculated with the fungus that the flaming orange crystals formed upon the bark surface amongst the aerial hyphae covering the twigs.

In his last paper on Phomopsis juniperovora (24, p. 901) the author made the following statement, "Single spore cultures from red cedar were observed which produced only a bare suggestion of the colour or lacked it entirely. These strains were classed as P. juniperovora, despite the abnormality in the colour character, on the basis of their origin on a cedar host in a locality where the typical colour-producing fungus was known to occur." With regard to this statement the author is now in a position to contribute further information. There does seem to be a tendency for certain forms of P. juniperovora to produce the empire yellow colour referred to, only in a slight degree. Such was the case of form no. 43968, recently isolated from diseased Juniperus virginiana from Rhode Island. This form was capable of producing the flaming orange crystals. With regard to the forms referred to in the quoted statement where the empire yellow colour was lacking entirely, the writer was dealing with P. occulta, a fungus which also occurs on Juniper, but which can now be differentiated/

differentiated from the parasite, P. juniperovora. In this respect it would be indeed interesting to know if Bottomley's Phomopsis on Cupressus in South Africa, might not possibly be a physiological form of P. occulta, which was parasitic in that particular locality. Bottomley did not report the yellow colouration in the medium, or the flaming orange crystals.

On Corn Meal Sugar Agar. Growth was at first appressed, silky, colourless, fairly rapid; aerial hyphae arising at the periphery of the colony; aerial stratum, vigorous, cottony, at first whitish but becoming tinged with orange colour, after the empire-yellow colouration had developed in the substratum below; flaming orange crystals developed in the mid stratum; with age the yellow colour deepened becoming orange-rufous. When freshly isolated the forms generally formed scant fruiting bodies, variously shaped, compound structures, from which the spores oozed in whitish or yellowish tendrils. Amongst the aerial hyphae ~~variously coloured~~ beads of moisture formed, - colourless, amber or ruby coloured.

Practically the same type of culture growth characteristics were obtained on oat -# and on Leonian's agars. The best fruiting occurred on the former, where it was observed that dark meandering lines formed in the pellicle of the colony growth along the sides of the test tube, particularly at the base of the slant. These dark lines circumscribed patches of hyphal growth/

growth in which stromata were observed to form.

The perfect stage was sought in these stromata but only the imperfect stage was found.

3. PHOMOPSIS CONORUM, (Sacc.) Died., Mich. II, 1882, p. 615; Sacc. Syll. Fung. III, 1884, p. 150; Ann. Mycol. IX, 1911, p. 22; Sacc. Syll. Fung. XXII, 1913, p. 903; Trans. Britt. Myc. Soc. XIII, 1928, p. 278.

Syn: Phoma conorum, Sacc. (1882)
Phomopsis conorum, Died. var. naviculisporea Trav. (1912).
 Bull. n° 8. Societe Flore Valdôtaine, p. 27, extr.
Phomopsis pitya, (Sacc.) Lind., pro. parte; (1913); nec. Phoma pitya, Sacc.
Phomopsis conorum, Sacc. v. H. (1917).

HISTORY OF THE FUNGUS.

Phomopsis conorum, (Sacc.) Died. was originally described ^{by} SACCARDO (54, p. 615) in 1882, as Phoma conorum on the cone scales of spruce. The description was very meagre and is apparently based on two specimens collected by Mlle. Libert (Lb. 291, 295). In 1901, SACCARDO (56, III, p. 150) restated this description and gave both Germany and France as the habitat of the fungus.

In his discussion of the genus Phomopsis DIEDICKE (13, p. 22) made the new combination P. conorum, (Sacc.), but he did not give further morphological detail regarding the fungus. SACCARDO in 1913, (56, XXII, p. 903) gave an amended description of this organism.

In 1917, von HÖHNEL (33) had suggested that Phomopsis Thujae, Died. was only a form of P. occulta, (Sacc.) or "P. conorum (Sacc.) v. H." We now know that P. Thujae is a form of P. occulta. Evidently "von Höhnel" as an authority for the combination P. conorum is/

is a misquotation. Otherwise, the combination P. conorum, (Sacc.) Died. is to be preferred on the basis of priority.

Phomopsis conorum and P. occulta have been confused by mycologists. The present investigation has shown that these two forms may be regarded as distinct and they are now readily distinguishable both morphologically and physiologically. Both species occur on all plant parts, - cones, stems, leaves and trunks. In the minds of the older mycologists there appears to have been a tendency to segregate fungus forms on cones from those occurring on conifer stems. Such a conception would needlessly tend to increase the species number.

In a paper recently published (25) the writer has shown that the well known fungus Phoma pitya, Sacc. discussed by Rostrup, who figured it in his *Plantepatologie* (52 p.568 fig.239) as an attributed parasite causing die-back of the terminals of Douglas fir and other conifers, and later regarded by Lind as Phomopsis pitya, (Sacc.), is no other than Phomopsis conorum (in part, for other fungus species were found among Rostrup's collections of P. pitya). It is perhaps to be regretted that an organism should bear the specific name conorum, when it is also stem inhabiting, and furthermore, when it does not occur within the life-history of Diaporthe conorum.

EXAMINATION OF EXSICCATI.

no. 1727. Phoma (Diaporthe) conorum, Sacc., on spruce
cone scales, Reliq. Lb. (Herb
Brussels).

This fungus is Phomopsis conorum.

no. ? Phoma (Diap.) conorum, spruce cone scales,
coll. Lb. (Herb. Sacc., Padua).

This fungus is Phomopsis occulta, Trav.

Phoma pitya, Sacc, Abies balsamea, Denmark
coll. H. Schested, 1883.

" " Pseudotsuga Douglasii
coll. ER., 1883.

" " Pseudotsuga Douglasii
1889.

" " Pseudotsuga Douglasii
coll. ER. (c.?)

These fungi in Rostrup's Herbarium
(Copenhagen) are all Phomopsis conorum.

DESCRIPTION OF THE FUNGUS.

Phomopsis conorum (Sacc.) Died.

Pycnidia ectostromatic, without a black line circumscribing the stroma, scattered or aggregate, simple or compound, arising within and seated upon the cells of the upper cortical tissue or the host amongst whose cells the fruit body is more or less incorporated; partially erumpent; black, carbonaceous, cone-shaped, lenticular, subglobose or truncate with with or without a definite ostiole; inner pycnidium of simple fruit body formed from a single primordium, unilocular, a cavity formed in one plane with a thickened layer of pseudoparenchymatous tissue above, (Pl. XIII, Fig. 3) cavity with protrusions from the sidewalls, hymenium lining the cavity more or less convoluted giving rise to sporophores; compound pycnidium formed by the fusion of several primordia, multilocular, chambers also tending to form and fuse in one plane, also forming above each other, 0.1-2.0 x 0.1-0.8 mm. Spores of three types, A, B and intermediate: A type, hyaline, unicellular, generally spindle shaped with acute or subacute gently rounded extremities, extreme range, (420), 6.2-14.6 x 2.5-4.7 μ , commonly, (300), 7.5-12.4 x 2.5-3.7 μ , with one, two or occasionally three oil drops, (Pl. X, Fig. 1); B type, hyaline, unicellular, filamentous, pronouncedly curved, hamate, horseshoe shaped, rarely straight, extreme range, (154), 15.5-34.4 x 1 μ , commonly (154), 20.2-24.1 x 1 μ ; with several small guttules (Pl. X, Fig. 2); intermediate spores, rarely/

rarely occurring, intergrading between the A and B types, very irregular in shape, attenuated, approaching the filamentous type, extreme range (50), 11.8-18.0 x 1.2-2.5 μ , commonly (50) 13.0-15.5 x 1.6-2.2 μ , (Pl. X, Fig.3); Sporophores, flexuous, subulate, with tapering acute or subacute extremities persistent; both A and B type spores produced on the same type of sporophore. extreme range, 5.6-21.4 x 1.0-2.5 μ , (Pl. X, Figs. 4 & 5). The spores are exuded in a whitish or yellowish tendril.

Perfect stage unknown.

Hab. According to Saccardo on the cone scales of Picea excelsa in France and Germany; according to Traverso on the same host in Italy. The fungus was identified by the writer among collections of fungi on Pseudotsuga Douglasii and Abies balsamea made in Denmark by Rostrup; Lind (Danish Fungi, p. 421) listed the fungus on cone scales of P. excelsa in Denmark (Seeland).

The fungus has been collected by the author on the following: in Scotland, on dead parts of living plants or P. Douglasii, and Picea Sitchensis, trunk of dead tree of Pinus Strobus, fallen cones of P. excelsa; in England, on frosted nursery transplant stock, P. Sitchensis; in Holland, on dead parts nursery transplant stock, P. Douglasii.

SPORE SIZE OF PHOMOPSIS CONORUM.

Variation of A Spore Size in Nature.

A measurement study of 280 A spores of Phomopsis conorum, representing 13 forms of species on Pseudotsuga Douglasii from Great Britain and the continent, showed a spore size range which at first appeared to be specific. When A spores of forms of the same species were studied with regard to the character on another host, Picea, (Table XXIII) variation in size was noted, but this variation was within the range determined for the Douglas fir forms.

Another form was discovered on Pinus Strobus which appeared to be the same species but on a new generic host. This form produced larger spores, but these were typical in shape. The inclusion of this form, therefore, would tend to extend the spore range of the species, Phomopsis conorum. At first it was thought that the Weymouth pine strain might be possibly distinct physiologically, and to determine this point the fungus was isolated in pure culture. The culture growth characteristics of the new strain from pine were identical with those of the Douglas fir and spruce forms on the same media. The fact that the P. Strobus potentially was able to produce small A spores in culture, will be brought out in the next section in the discussion of spores of D. conorum produced in culture.

<u>Picea Sitchensis</u>	43917 *	S. Fifehire	50	9.0	x	3.1	6.2-12.1 x 2.5-4.0
" "	43983	England, Oxford	60	10.8	x	2.9	8.7-13.0 x 2.5-3.4
" <u>excelsa</u> - cone	Wilson coll.	S. Stirlingshire	10	11.2	x	3.0	9.9-12.4 x 2.5-3.4
Total	(3 coll)		120	10.1	x	3.0	6.2-13.0 x 2.5-4.0

<u>Pinus Strobus</u>	43980 *	S. Peeblesshire	20	12.2	x	3.4	9.9-14.6 x 2.8-4.7
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* Forms in culture; culture growth characteristics identical throughout.

TABLE XXIII.

COMPARISON: A SPORES OF P. CONORUM ON THREE GENERIC HOSTS.

Host.	No. Collec- tion.	Source.	No. of Spores.	Average, length x width, μ	Extreme Range length x width, μ
<i>Pseudotsuga Douglasii</i>	43920	Scotland, Fifeshire	10	9.6 x 3.3	8.1-12.4 x 2.8-3.7
"	43922 *	S. Roxburghshire	10	9.2 x 3.4	8.1-11.5 x 2.8-4.0
"	43926	S. Fifeshire	10	10.0 x 3.1	8.4-11.2 x 2.5-3.4
"	43927 *	"	50	8.8 x 2.8	6.8-12.4 x 2.5-3.4
"	43928 *	"	30	9.8 x 3.2	6.5-12.4 x 2.5-3.7
"	43929	"	10	9.6 x 3.1	7.8-10.9 x 2.8-2.4
"	43944	S. Peeblesshire	10	9.3 x 3.2	9.1-11.5 x 2.8-4.0
"	43946	S.	10	10.1 x 3.3	8.7-12.4 x 2.8-3.4
"	43959	Denmark, Lyngby	30	9.5 x 3.1	6.5-12.4 x 2.8-3.4
"	43960	Holland, Schovenhorst	20	9.5 x 3.0	7.4-11.6 x 2.8-3.4
"	43962 *	S. Peeblesshire	50	9.7 x 3.0	8.1-11.8 x 2.5-3.7
"	43963 *	Wales, Lake Vyrnwy	30	8.9 x 3.4	6.2-11.2 x 2.8-4.0
"	43983 *	S. Peeblesshire	10	11.5 x 3.5	9.9-13.6 x 2.8-3.7
Total	(13 coll.)		280	9.5 x 3.1	6.2-13.6 x 2.5-4.0

Table XIII presents the variation in spore size obtained on three generic hosts. It will be noted that the forms studied from Douglas fir agreed markedly with those on Picea so far as range is concerned; the average length variation among the forms of the two generic host groups was likewise very similar. When the spores of the form isolated from pine were considered they were observed to extend beyond the upper part of the range, determined for forms on Douglas fir and spruce, thereby extending the spore range for the species. The Table is important for it brings out how essential it becomes to have a more complete knowledge of a given fungus species, with regard to the extent of variation of spores size throughout the entire host range of the fungus.

Variation of A Spore Size in Culture.

Phomopsis conorum did not produce A spores vigorously in culture as did P. occulta. Only four of the eight forms of P. conorum isolated for life-history study, reproduced spores. On natural media ^hwhere one would ordinarily expect abundant sporulating ^hfructifications, pycnidiospores, particularly the A type, formed slowly. B spores generally formed, a scant number of the A type occurring amongst these. In such cases the A spores were inclined to be much shorter and were produced at the lower part of the spore range determined for naturally produced spores.

A comparison between culturally and naturally produced spores is brought in Table XXIV. It will be noted that in the one case where an abundance of A spores formed, (no. 43962 B⁴), Douglas fir form on sugar-corn meal agar) the spores were correspondingly larger than those which occurred amongst a preponderance of the B type. The artificially produced A spores of this strain were practically identical in size with spores produced in nature on the Douglas fir.

In the case of the Weymouth pine form, the culture-produced spores were considerably shorter than those found in nature. The average and range were close to that of naturally-produced spores of forms on Douglas fir (Table XXIII). It would seem to indicate therefore, that the pine fungus tended to form longer spores. Further tests may determine such to be the case, in which instance, the form may be regarded as a separate physiological strain.

Variation of B Spore Size in Nature and Culture.

In nature Phomopsis conorum produced both A and B spores readily. The latter were very difficult to measure because of the extremely curved shape of the spores. By careful search, however, spores were found which were inclined to be straight, and these were selected for the measurement study. Spore width, which is approximately 1 μ , was not considered in the investigation. A measurement study of 154 B spores/

TABLE XXIV.
1

A SPORES OF PHOMOPSIS CONORUM IN NATURE AND CULTURE COMPARED.

(1).

Natural Host and Culture Substratum	No. Fungus	No. Spores	Average, length x width, μ	Extreme Range, length x width, μ
<u>Picea Sitchensis</u>	43917	50	9.0 x 3.1	6.2-12.1 x 2.5-4.0
Elm twig	" C ¹	4	7.7 x 3.3	7.1- 8.7 x 2.8-3.4
<u>Pseudotsuga Douglasii</u>	43928	30	9.8 x 3.2	6.5-12.4 x 2.5-3.7
" twig	" B ⁴	20	8.8 x 3.2	6.5- 9.6 x 2.8-4.0
<u>P. Douglasii</u>	43962	50	9.7 x 3.0	8.1-11.8 x 2.5-3.7
Sugar-corn meal agar	" B ⁴	20	9.6 x 3.0	7.8-11.8 x 2.5-4.0
Oat agar + 2% Taka-diastase (2)	" F ⁷	20	8.9 x 3.1	7.5-12.4 x 2.5-3.4
<u>Pinus Strobus</u>	43980		12.2 x 3.4	9.9-14.6 x 2.8-4.7
Elm twig	" C ²	20	9.3 x 3.2	7.8-11.2 x 2.8-3.7

(1).

A spores were produced only scantily, with an abundance of B spores, except in the case of 43962 B⁴.

(2).

Taka-diastase, 2% sol., did not stimulate the production of an abundance of A spores.

spores from 12 forms of the species on Douglas fir, spruce and pine, indicated variation in size within a given range. Eighty spores produced in culture by 8 isolated forms on hard agars and twigs, both of conifer and broad-leaved hosts, showed satisfactory agreement with naturally-produced B spores. The average spore length of culturally-produced spores was somewhat greater. In this connection it was observed that the B spores tended to be slightly longer when produced in great abundance, and with only a scant number of A spores. The averages and ranges of this comparison are given in Table XXV.

TABLE XXV.

LENGTH AVERAGES, AVERAGE AND EXTREME RANGES
OF B SPORES IN CULTURE AND NATURE, COMPARED.

Nature or Culture	No. Spores	Average μ	Average range, μ	Extreme range, μ
Nature	154	22.2	20.2 - 24.3	15.5 - 34.4
Culture	80	24.2	22.2 - 26.9	17.7 - 34.1

Variation of Intermediate Spores in Nature and Culture.

Intermediate spores were not found nearly so abundantly in nature in the life-history of Phomopsis conorum, as they were in the life-history of P. occulta. A measurement study of 50 spores found in seven collections of the species, indicated a specific range, $11.8-17.4 \times 1.2-2.5\mu$, intermediate between that of the A and B types. A scant number of intermediate spores were found in only one culture, which produced mostly A spores. The range of these spores was found to be within that of intermediate spores produced in nature.

SPORE SHAPE IN NATURE AND CULTURE.

The typical spindle shaped, more or less pointed, spore which predominated in collections of the species Phomopsis conorum in nature, was also found in culture. The shape of the B spores was also constant in both nature and in culture. At the outset of the study of the species, when the pronouncedly curved shape of the filamentous spores was first observed, considerable interest was awakened at the time, to observe whether or no, this character of curvature was constant. Invariably one found it in nature; similar hooked or horseshoe shaped were likewise obtained in culture. The character of curvature must therefore be considered inherent in the species. In diagnosing the species, it will undoubtedly prove a most helpful character.

CULTURAL LIFE-HISTORY OF P. CONORUM.

The perfect stage of Phomopsis conorum is unknown. Experiments were performed for the purpose of obtaining this stage in culture, but only negative results were obtained. In nature Diaporthe conorum was observed growing on the same stem of Sitka spruce as the P. conorum. Monoascospores isolations of the D. conorum, as has been previously stated, produced only the imperfect stage, P. occulta in culture. Evidently P. conorum does not belong in the life-history of this Diaporthe.

Sterile twigs of Alnus, Ulmus, Pseudotsuga, were inoculated May 30, 1927, with culture strains of forms of P. conorum isolated from Douglas fir. When growth had started the twigs were placed in cold storage, and the temperature kept approximately freezing. After three months the twigs were brought back to room temperature, and kept under moist conditions. The perfect stage has not been observed. Sterile stromatic, pulvinate, or otherwise polymorphic bodies formed, 1-5 mm. broad, 1-5 mm. high. In a few instances the imperfect stage was observed to form amongst these bodies, but the spores were the B type generally. A spores occurred rarely and after a longer period of time.

Cultures of eight forms of P. conorum isolated from three generic hosts have been observed closely/

closely in a series of sub-cultures on the three hard media employed. But the perfect stage did not appear. The cultures have been kept out of doors in a cold greenhouse in full and in partial sunlight, as well as at ordinary room temperature.

Form no. 43962, isolated from Douglas fir showed a greater tendency to form the imperfect stage, than the other forms studied. Accordingly, cultures of this strain growing on oat agar were treated with the ferment Taka-diastase, - 0.5 cc. of 1%, 2%, 4%, 6%, respectively. The imperfect stage was obtained only in the tubes treated with the 1% and 2% solutions.

CULTURE GROWTH CHARACTERISTICS.

Phomopsis conorum grew readily upon the three media employed. Vegetative production of aerial hyphae was most luxuriant on the hard oat agar. Upon this medium there was a tendency to form large, compound stromata which occasionally produced locules containing spores. Along the pellicle dark lines circumscribing areas of fungal tissue appeared. Vegetative growth of hyphae was inclined to be heavy on twigs of natural media. Patches or russet-green colour appeared, a colour which was noted on Leonian's agar. Curiously shaped polymorphic fruiting structures were obtained with form no. 43928 on a twig of Douglas fir in culture (Pl. XI, Fig. 1).

On Sugar-Corn Meal Agar. Growth at first was fairly rapid, silky, appressed; aerial hyphae arose at the advancing edge of the colony, which became distinctly zonate. This zonation was observed to occur both in the dark and in the light; aerial hyphae, whitish overlying the midstratum in which a dull olivaceous colour appeared, which rapidly became blackish. Large, irregular, scattered stromata formed at the base of the tube.

On Leonian's Agar. Growth characteristics on this medium resembled those on sugar-corn meal agar. At first the aerial stratum consisted of short, dense, powdery, whitish hyphae overlying a colourless appressed midstratum. During the first week of colony growth zonations/

zonations formed which became very pronounced as the colony aged. There also appeared at this time a slight production of a dull olive-green colour in the midstratum which tended to become blackish. Patches of russet-green colour occasionally appeared amongst the aerial growth. Stromata formed most sparingly. Spores were not obtained on this medium. (Pl. XXIII).

4. Phomopsis Montanensis, n.sp.HISTORY of the FUNGUS.

In 1927, material of diseased branches of Abies grandis, Lindl. and Abies lasiocarpa, Nutt. collected by Mr C.R.Stillinger in Montana was sent to the writer by Dr.J.S.Boyce for identification. Stillinger reported that dead 'flags' associated with the fungus were abundant on all age classes of the A.lasiocarpa, and that in some cases, twenty-five per cent of the branches were killed. Upon a diagnosis of ^{the} Montana material it was found that Phomopsis Boycei occurred on the diseased branches of the great fir (See p.213.) which agreed with material previously sent to the writer from Idaho, for examination.

a. Phomopsis sp., not found on Abies grandis, was discovered on the alpine fir, which showed marked morphological differences from P.Boycei. This species is being provisionally described as new, inasmuch as it appears to be quite distinct from the other species of the genus dealt with in this paper.

DESCRIPTION/

DESCRIPTION OF THE FUNGUS.

Phomopsis Montanensis n.sp.

Pyenidia ectostromatic, scattered or aggregate, simple or compound, arising within and seated upon the cells of the upper cortical tissue of the host, amongst whose cells the fruit body is more or less incorporated; partially erumpent; black, carbonaceous, cone-shaped, lenticular, sub-globose, or truncate, breaking open with an irregular orifice; inner pycnidium of simple fruit body formed from a single primordium, - unilocular, in smaller primordia, cavity subspherical or elliptical with a thickened layer of pseudoparenchymatous tissue above, larger primordia distinctly multilocular, chambers tending to fuse forming an irregularly shaped cavity, lined with a convoluted hymenium; compound pycnidia formed by the fusion of several primordia, distinctly multilocular, .01-0.5x.01-.4 mm. (Pl. XVII, Fig. 1); spores of two types, A and B: A type, hyaline, unicellular, generally oblong, elliptical, or ovate with obtuse extremities, extreme range (100), $5.3-8.1 \times 2.2-3.4 \mu$, commonly, (100). $6.0-7.8 \times 2.5-3.4 \mu$, average, (100) $6.9-2.8 \mu$, with two guttules, (Pl. XIV, Fig. 1); B type, hyaline, unicellular, filamentous, cylindrical, slightly/

slightly curved or almost straight, occurring rarely, extreme range (20) 9.3-11.8 x 0.9 μ , average, 10.5 x 0.9 μ , with several small guttules (Pl. XIV, fig. 2) sporophores, short, delicate (Pl. XIV, figs. 3 & 4) subulate, extreme range, (20), 4.7-11.1 x 0.9-3.1 μ ; spores exuded in a whitish or yellowish tendril. (1).

The perfect stage is unknown.

Hab. On dead branches and twigs of Abies lasiocarpa in Montana. Type specimen, No. 43987, collected by C. R. Stilling, on dead branches of A. lasiocarpa,

Aug. 24, 1927, Belton, Montana, U.S.A.

(1).

Phomopsis Montanensis, n. sp.

Pycnidiis discretis vel aggregatis, sub initio subepidermicis denique erumpentibus; carbonaceis, lenticularibus, conoideis, truncatis, vel subglobosis, ostioli paulo pertusis, vel deficientibus, contextu heterogeneo praeditis, matricibus fuliginosis supra cavitates sporiferas incrassatulis; pycnidiis parvis, majoribus multilocularibus vel per dissepimentum disruptionem unilocularibus, ceteris unilocularibus; .01-.5 x .01-.4 mm. Sporulis dimorphis; A sporulis, hyalinis, continuis, oblongis ad ovatis, eguttulatis, (100) 5.3-8.1 x 2.2-3.4 μ , vulgo, (100) 6.0-7.8 x 2.5-3.4 μ ; B sporulis filiformibus, leviter curvatis, eguttulatis minutis, (20) 9.3-11.8 x 0.9 μ ; basidiis continuis, subulatis, brevis, tenuis, 4.7-11.1 x 0.9-3.1 μ .

Hab. in ramis emortuis Abietis lasiocarpa, Nutt. in America boreali (Montana). August, 1927.

SPORE SIZE AND SHAPE OF *P. MONTANENSIS* IN
NATURE AND IN CULTURE.

The close agreement between the average size of A. spores produced in culture, with those produced in nature, is brought out in TABLE XXVI

Spores were produced on Leonian's agar within a month, from monospore isolations. B. spores were not observed in culture so a comparison could not be made with this type. The typical oblong elliptic or ovate spores with obtuse extremities generally observed in the type specimen were also produced culturally.

(TABLE XXVI) SIZE AVERAGE and RANGE of A. SPORES of P. MONTANENSIS PRODUCED in NATURE and in CULTURE, COMPARED.

NATURE or CULTURE	NO. SPORES	AVERAGE LENGTH X WIDTH, μ.	EXTREME RANGE LENGTH X WIDTH, μ.
<u>Abies lasiocarpa</u> (nature)	100	6.9 - 2.8	5.3-9.3 x 2.2-3.4
Leóniens' agar	50	6.8 - 2.7	5.6-8.1 x 2.2-3.4

COMPARISON of P. MONTANENSIS with P.PSEUDOTSUGAE
&
P. STROBI.

A preliminary examination of Phomopsis Montanensis showed it to be quite distinct from P.Boycei on Abies grandis. The newly discovered species seemed most closely to resemble P.Pseudotsugae and P.Strobi. (See p.190 and p. 183). The culture growth characteristics were distinct however, differentiating it from both of these species, physiologically.

A biometrical study of 100 spores of the type specimen was made. In TABLE XXVII is given a comparison of P.Pseudotsugae and P.Strobi with P.Montanensis, which indicates a significant difference between spore populations of P.Montanensis and P.Strobi; no difference is shown between P.Pseudotsugae and the new species.

(TABLE XXVII) BIOMETRIC CONSTANTS of P. PSEUDOTSUGAE, P. STROBI (TYPE)
P. STROBI (ABIES PECTINATA, ITALY), and P. MONTANENSIS, COMPARED

LENGTH

POPULATION	NO. SPORES	M	SIGNIF. OF MEAN DIFFERENCES SIGNIF. from	STANDARD DEVIATION	COEFFICIENT of VARIABILITY
<u>P. Montanensis</u> (type)	100	6.9 ±.052		.767 ±.037	11.12 ±.537
<u>P. Pseudotsugae</u>	200	6.7 ±.074	none	1.56 ±.053	23.29 ±.827
<u>P. Strobi</u> (type)	100	5.8 ±.033	Signif. from	4.88 ±.023	8.41 ±.404
<u>P. Strobi</u> (<u>A. pectinata</u> Italy)	100	5.8 ±.051	Signif. from	7.61 ±.036	13.12 ±.636

WIDTH

<u>P. Montanensis</u> (type)	100	2.9 ±.020		.302 ±.014	10.40 ±.501
<u>P. Pseudotsugae</u>	200	2.6 ±.022	none	.466 ±.016	17.93 ±.624
<u>P. Strobi</u> (type)	100	2.3 ±.023	Signif. from	.335 ±.016	14.56 ±.709
<u>P. Strobi</u> (Italy)	100	2.3 ±.033	Signif. from	.487 ±.023	21.17 ±1.054

Although a significant difference did not exist between Phomopsis Montanensis and P.Pseudotsugae other morphological differences tended to separate the Douglas fir organism as a distinct species. B spores which were described for the fungus on Abies lasiocarpa are not known to occur in the life-history of P.Pseudotsugae. In shape the spores of the two species were quite distinct; for those of P.Montanensis were typically oblong, elliptic or ovate, whereas those of P.Pseudotsugae were elliptic fusoid. Physiologically the two species were quite distinct.

CULTURE GROWTH CHARACTERISTICS:

The culture growth characteristics of Phomopsis Montanensis proved to be constant throughout several culture generations.

On/

On sugar-corn-meal agar. Growth was slow, strongly appressed, colourless. Within a week, flocculent short, aerial hyphae developed about the inoculum, in the neighbourhood of which an olivaceous colour appeared in the midstratum, along with abundant primordia. (This green colour was of the same intensity as that formed by P.Pseudotsugae in the same medium).

By the end of two weeks abundant simple and compound pycnidia studded the surface of the colony for a radius of 10 mm. The colony eventually staled, the hyphae at the periphery becoming olivaceous in colour and growing down into the substratum which was tinged a dark brownish colour; the aerial growth became felt-like and grayish.

Growth characters on Leonian's agar were identical with those on sugar-corn meal agar.

5. Phomopsis Strobi Syd.

Ann. Myc. XX, 1922, p.204.

HISTORY of the FUNGUS.

Phomopsis Strobi was described by SYDOW (65) as a new species from material collected by WEIR on limbs of Pinus Strobus in the United States (Maine, 1918). The fungus occurred in association with Peridermium Strobi Kleb. With regard to this fungus SYDOW stated,- "The fungus can evidently be regarded only as a Phomopsis. It differs essentially from the essential form of the genus only by the delicate conidiophores. We were not able to identify the form with other of the numerous forms described on conifers. Phoma pini Cke. et Hark. (Sacc.Syll.Fung.III.p.73) appears to be another fungus."

DESCRIPTION of the FUNGUS,

Phomopsis Strobi Syd.

Pycnidia ectostromatic, scattered or sparsely aggregate, simple or compound, arising within/

in and seated upon the cells of the upper cortical tissues, amongst whose cells the fruit body is more or less incorporated; partially erumpent, black carbonaceous, cone-shaped, sub-globose, breaking open irregularly at the apex; inner pycnidium of simple fruit body formed from a single primordium, - unilocular, cavity more or less spherical or elliptical without a heavy development of pseudoparenchymatous tissue above, larger primordia, multilocular, with numerous, fully formed or incomplete chambers of various shape and size (.2-.25 mm.diam.) which tend to fuse becoming irregularly unilocular with convoluted hymenium, walls of the pycnidium thin at the sides, and upon the floor of the fruit body, 12-15 μ , composed of hyaline and yellowish coloured elongated cells; compound primordia formed by the fusion of several primordia, distinctly multilocular, .45-.6 x .3-.4 mm.; Spores of one type A-type, hyaline, unicellular, generally short, oblong elliptic, but varying to elliptic-fusoid, extremities obtuse, symmetrical or asymmetrical, extreme range (100) 4.7-7.1 x 1.9-3.1 μ , commonly (100), 5-6.5 x 1.9-2.8 μ ; with two guttules (Pl.XV, Figs 1 & 3); sporophores (Pl.XV, Figs. 2 & 4) very short, delicate, subulate, 4.5-8.1 (10) x 0.6-1.2 μ . Perfect stage unknown.

EXAMINATION/

EXAMINATION OF EXSICCATI.

NO. 10898. Phomopsis Strobi Syd., on Pinus Strobus
Coll. Weir, U.S., (Maine, 1918), portion of type
preserved at Herb.Syd., Berlin.

Specimens on Pinus montana and P.Stro-
bus, collected by ROSTRUP, and identified by him as
Phoma pitya Sacc. (1884) were examined by the author
(25, p.280) and found to be forms of Phomopsis
Strobi.

COMPARISON of PHOMOPSIS STROBI with P.PSEUDO-
TSUGAE and FORMS on PINUS and ABIES in EUROPE.

Only one form of Phomopsis Strobi (the
type specimen) was studied intensively, with regard
to spore size. The average length and width obtain-
ed by measurements of 100 spores agreed with those
of the two forms on Pinus Strobus and P.Montana
discovered in ROSTRUP'S herbarium (25),
identified as Phoma pitya; the range of the Danish
collections/

collections was within that of the spore range of the type. In shape, also ROSTRUP'S specimens agreed with that of Sydow's P.Strobi.

Spores of the type of Phomopsis Strobi were also compared in shape and size with a form of Phomopsis on Abies pectinata, collected by Petri in Italy, cultures of which have been received from him for comparison with forms of Phomopsis abietina from the same host. Culturally Petri's Phomopsis differed from all the Phomopsis forms investigated in this paper; morphologically, it resembled most of all Phomopsis Pseudotsugae, and P.Strobi.

A comparison was made of the biometrical constants of Phomopsis Strobi (type), and the culture form of the Phomopsis sp. (on oat agar) from Italy, together with Phomopsis Pseudotsugae. This comparison is presented in TABLE XXVIII.

A study of TABLE XXVIII shows that the only significant difference which occurs is between the constants for length of Phomopsis Strobi and P.Pseudotsugae, the former being a shorter spored form. Until it is possible to obtain fresh material of P.Strobi from the U.S. for culture experiments, it is assumed that P.Strobi and the Phomopsis sp. obtained/
ed/

(TABLE XXVIII) BIOMETRICAL CONSTANTS of P. STROBI, PHOMOPSIS SP. (PETRI) and P. PSEUDOTSUGAE, COMPARED.

POPULATION	NO. SPORES	M	LENGTH		STANDARD DEVIATION	COEFFICIENT of VARIABILITY
			SIGNIF. of MEAN DIFFERENCES SIGNIF. from	SIGNIF. from		
<u>P. Strobi</u> (type)	100	5.8 ±.033			.488 ±.023	8.41 ±.404
<u>Phomopsis Sp.</u> (Petri)	100	5.8 ±.051	none		.761 ±.036	13.12 ±.636
<u>P. Pseudotsugae</u>	200	6.7 ±.074	Signif. from		1.56 ±.053	23.29 ±.827
			WIDTH			
<u>P. Strobi</u> (type)	100	2.3 ±.023			.335 ±.016	14.56 ±.709
<u>Phomopsis Sp.</u> (Petri)	100	2.3 ±.033	none		.487 ±.023	21.17 ±1.054
<u>P. Pseudotsugae</u>	200	2.6 ±.022	none		.466 ±.016	17.93 ±.624

obtained from Petri are identical. If such an assumption is true, then P.Pseudotsugae is distinct culturally from P.Strobi. This cultural difference, together with morphological differences in size and in shape, would tend to separate the two forms as distinct species. It is admitted, however, that the relationship between the two is very close.

CULTURE GROWTH CHARACTERISTICS.

The culture growth characteristics of Phomopsis Strobi herein given, were those obtained by/culture studies of a Phomopsis form, isolated by Petri from Abies pectinata, in Italy. This form, as has already been stated, is regarded provisionally as a form of P.Strobi. The culture characteristics were quite distinct from those of P.Pseudotsugae, and from P.abietina, which organism, it was thought for a time, the Italian form might possibly (be due to) the host relationship of the organism ().

The Italian form of Phomopsis Strobi was first studied in culture, December, 1927. Cultures on oat agar, kept at ordinary room temperature, produced an abundance of pycnidia and spores within three weeks. In shape the spores were distinct from those/

possibly, because
g

those of both Wilson's and Hartig's organisms. The form obtained from Petri has been in culture now for approximately one year; as yet no diminution of vigour of growth, or spore production, has been observed.

On sugar-corn meal agar. Growth was at first rapid, appressed, colourless and silky. An olive brown colour appeared in the mid stratum staining the medium, this colour continued to keep ~~a~~pace with, but to form just behind the advancing edge of the colony. The aerial stratum had a fine, granular appearance, indistinctly regularly zonate. Within two weeks the surface about the centre of the colony was covered with abundant pycnidia simple and compound, which were oozing spores abundantly by the third week. During this period the olive brown colour in the midstratum and throughout the medium had darkened considerably, becoming dull olivaceous. The fine granular character of the aerial hyphae, which became grayish, was persistent.

The growth characteristics on Leonian's agar, were identical with those on the medium just described. Fruiting was, likewise abundant on Leonian's agar. (Pl. XXIV, Fig. 4).

6. PHOMOPSIS PSEUDOTSUGAE, Wilson.

Trans. Proc. Bot. Soc. Edin. XXVIII, 1920, p.47;
 For. Com. Bull. 6, 1925, p.9; Trans. Brit. Myc.
 Soc., XIII. 1928, p. 273.

HISTORY OF THE FUNGUS.

Phomopsis Pseudotsugae was first described by WILSON (75) in 1920, and later more fully in 1925, (76). Wilson attributed a die-back and canker disease of the Douglas fir (Pseudotsuga Douglasii) to this organism, which he discussed and figured in detail in the later publication.

The relationships of Phomopsis Pseudotsugae have been stated both by WILSON (76) and by BOYCE (4). On account of the confusion in the literature due to a lack of knowledge concerning the actual identity of Phoma pitya, Sacc. and of Phoma abietina, Hart., a state of affairs was reached wherein the names P. pitya and P. abietina were regarded by some authorities as interchangeable, and were used in certain cases indiscriminately, to indicate the fungi concerned. At the same time P. Pseudotsugae was also being confused with these two species. To clear up matters and investigation of the type specimen of P. pitya was undertaken, together with that of authentic specimens of P. abietina. The results of this investigation, which proved the separate identity of these three organisms, were published recently by WILSON and HAHN (77, 25).

Phomopsis/

Phomopsis Pseudotsugae appears to be a good species. It seems to be most closely related to P. Strobi but several morphological, as well as physiological differences, separate these two species. P. Pseudotsugae is known only in Europe. Whether it exists in America on the Douglas fir is debatable. Considering the large amount of importation of conifers on that side of the Atlantic from Europe, which are hosts for the species it would not be surprising if the organism was found there. As yet typical P. Pseudotsugae has not been discovered in North America.

(1).

DESCRIPTION OF THE FUNGUS.

Phomopsis Pseudotsugae, Wilson.

Pycnidia ectostromatic, scattered or aggregate, simple or compound, arising within and seated upon the cells of the upper cortical tissue of the host, amongst whose cells the fruit body is more or less incorporated; partially erumpent; black, carbonaceous, lenticular, obpyriform, subglobose, with or without a definite ostiole; inner pycnidium of simple fruit body formed from a single primordium, - smaller primordia unilocular, cavity subspherical or elliptical without a heavy development of pseudoparenchymatous tissue above, larger primordia, multilocular, cavities tending to fuse forming an irregular cavity lined with a convoluted hymenium; walls of pycnidium, thin at the sides and upon the floor of the fruiting body, composed of hyaline and yellowish coloured elongate cells, tissue above dark, olivaceous; large compound primordia formed by the fusion of several primordia, distinctly multilocular; .01-1.0 x .01-4.0 mm. (Pl. XVII, Figs, 2 & 3); spores of one type only, A type, hyaline, unicellular, typically elliptic-fusoid, varying in some to ovate or oblong, with subacute or obtuse extremities/

(1).

An amended description of Phomopsis Pseudotsugae was recently published (77). This description is repeated here, but with certain modifications. These changes are necessary on account of additional information secured by a further study of the species.

extremities (Pl.XVI, Fig. 1), occasional longish spores, elongate-elliptical, or cylindrical, with obtuse or subacute extremities (Pl.XVI, Fig. 2), extreme range, (210), $4.7-12.4 \times 1.9-3.4\mu$, commonly, (210), $5.3-9 \times 2.2-3.1\mu$, (average, 210 spores, 10 coll., $6.7 \times 2.6\mu$), occasionally with two guttules; sporophores short, delicate, subulate $4.7-10.5 \times 1.0-2.5\mu$, (Pl.XVI, Fig. 3); spores exuded in a single whitish tendril or droplet, or from compound pycnidia, in several tendrils emerging from different parts of the same fruit body.

Perfect stage unknown.

Hab. On branches, trunks and leaves of Pseudo-tsuga Douglasii in Great Britain, Ireland, Norway, Sweden, Denmark and Holland; also on branches of P. glauca, Larix europaea, L. leptolepis, L. Sibirica, Abies pectinata, Cedrus Atlantica, C. Deodara, and Sequoia gigantea.

SPORE SIZE OF P. PSEUDOTSUGAE.Variation of Spores in Nature.

A measurement study was made of 210 spores secured from ¹⁰collections of the fungus in Scotland and on the continent in Denmark, Sweden and Holland. Culturally the British forms showed excellent agreement with each other and with the continental forms.

In Table XXIX the spore size average and extreme range, is given for the various forms. It will be noted that three of the forms, nos. 43934, - 40, - 60, showed the greatest variation in spore size. Wilson gave the upper limit of the range for length, as 8.5μ . An extension of the upper spore range is therefore necessary. This particular measurement study, indicates, as it did in the case of P. conorum, the desirability ^{of} observing a considerable number of forms of a given species before attempting to limit its size range. Wilson regarded the species as fairly constant, showing little variability. That he was correct in this estimation of his species is brought out in the Table; Wilson gave the range of his species as $5.5-8.5 \times 2.5-4.0\mu$.

TABLE XXIX.

SPORES SIZE OF TEN FORMS OF *P. PSEUDOTSUGAE* ON DOUGLAS FIR
FROM GREAT BRITAIN AND THE CONTINENT, COMPARED.

Source.	No. fungus	No. spores	Average length x width, μ .	Extreme range, length x width, μ .
Scotland	38852 *	10	6.4 x 2.4	4.7-6.8 x 1.9-2.8
"	43919	10	5.9 x 2.5	5.6-6.2 x 2.2-2.8
"	43921 *	10	6.3 x 2.5	5.3-8.1 x 1.9-2.8
"	43934 *	25	7.0 x 2.7	5.3-9.6 x 2.2-3.1
"	43938 *	10	5.8 x 2.2	5.3-6.5 x 1.9-2.5
"	43942 *	65	7.2 x 2.6	4.7-12.4 x 2.2-3.4
Denmark	43958 *	10	6.0 x 2.3	5.6-7.1 x 2.2-2.5
Holland	43960 *	50	6.8 x 2.5	4.7-10.5 x 1.9-3.4
"	43961	10	6.5 x 2.5	5.6-7.1 x 2.2-3.1
Sweden	43988 *	10	6.0 x 3.0	4.7-6.8 x 2.5-3.1
Total	(10 coll.)	210	6.7 x 2.6	4.7-12.4 x 1.9-3.4

* In culture; these forms showed identical culture growth characteristics.

Variation of Spores in Culture.

Four forms of Phomopsis Pseudotsugae (nos. 38852, 43918, - 53, - 58) from Douglas fir were studied in culture with regard to size of spores. It was found that variation in this respect was extremely limited, the range being well within the confines of that determined for the species in nature. The average size of 50 culture-produced spores indicated an average slightly shorter and broader, than that of spores produced in nature. This average is given in Table XXX.

SHAPE OF SPORES OF P. PSEUDOTSUGAE IN NATURE
AND IN CULTURE.

In nature the spores of Phomopsis Pseudotsugae were typically elliptic-fusoid (Pl. XVI) with obtuse extremities. Variation in size occurred, however, certain of the spores becoming short-ovate or oblong-elliptic with obtuse extremities. The fusoid-elliptic type was not so pronounced in culture as in nature. The long-elliptical or cylindrical spores (Pl. XVI, Fig. 2) observed infrequently occurring in nature, were not found in culture.

SIZE AVERAGES AND RANGES OF
CULTURALLY AND NATURALLY PRODUCED SPORES OF P. PSEUDOTSUGAE, COMPARED.

TABLE XXX.

Nature or Culture	No.	Average Length x width, μ	Average length x width, μ	Extreme range, length x width, μ
Nature (10 forms)	210	6.7 x 2.6	5.8-7.2 x 2.2-3.0	4.7-12.4 x 1.9-3.4
Culture (4 forms)	50	6.2 x 2.8	6.1-6.2 x 2.7-2.9	5.3-9.3 x 2.2-3.4

CULTURAL LIFE-HISTORY OF P. PSEUDOTSUGAE.

In 1925, WILSON (77) reported the perfect stage of Phomopsis Pseudotsugae to be Diaporthe pitya, Sacc.. He sowed ascospores on Douglas fir decotion agar which produced a mycelium similar in appearance to that of P. Pseudotsugae, and in about three weeks' time pycnidia were produced containing the spores of this imperfect fungus borne on long sporophores.

The writer has not been able to repeat Wilson's results; for forms of Diaporthe conorum (= D. pitya) isolated in monoascospore culture produced only the spores of the imperfect stage Phomopsis occulta. Douglas fir decotion agar was not tested in these experiments, but inoculated twigs of Douglas fir produced P. occulta, abundantly. P. Pseudotsugae did not appear in the life-histories of any of the forms of D. conorum studied.

Experiments to obtain the perfect stage with forms of Phomopsis Pseudotsugae gave negative results. Only the imperfect stage was obtained in the following experiments which included:

- (1) Growth of P. Pseudotsugae on twigs of conifers (including Pseudotsuga Douglasii), and broad-leaved hosts, kept approximately at freezing temperature for three months and then brought back to room temperature where they were kept under moist conditions.

(2)/

- (2) Mixture of 26 culture forms of P. Pseudotsugae, obtained from single and poly-spore isolations, from different geographical sources (Great Britain and the continent), in oat agar tubes two cultures planted in a tube.
- (3) Addition of Taka-diastrase, 0.5 cc. of 1%, 2% 4%, 6%, respectively to four oat agar tubes of monopycnidiospore culture form, no. 43918, after vegetative growth of the fungus was well started.

CULTURAL GROWTH CHARACTERISTICS.

Phomopsis Pseudotsugae proved to be a species very constant with regard to its culture growth characteristics which were not only persistent but also manifest a general uniformity. This uniformity is demonstrated when forms were isolated from widely separated sources in Great Britain and on the continent, and from widely separated (phylogenetically) generic hosts. Thirty-three forms of the organism have been studied in culture isolated from Sequoia, Cedrus, Pseudotsuga and Larix

On Sugar-Corn Meal Agar. Growth was rapid at first colourless, appressed, silky. Within a week a green colour (olivaceous), developed in the midstratum about the inoculum, and extended practically out to the edge of the colony. Aerial growth was at first long, fibrous, whitish and irregular; this fibrous growth gradually became grayish and irregularly matted. In the moister portion of the tube large/

large, compound stromatic pycnidia formed which oozed spores in whitish or yellowish droplets. Smaller, simple pycnidia also were found in the drier parts of the slant. With age the green colour which had developed in the mid-and substrata became **dulled**, assuming a darkish brown caste.

Culture characteristics on Leonian's agar were practically identical with those on sugar-corn meal, except for the fact that on the former medium, fruiting was very scant. On oat agar the fungus fruited vigorously. (Pl. XXIV, Fig. 3).

Three forms of Phomopsis Pseudotsugae have been maintained in culture, for approximately six years. They still continue to produce fruiting bodies and the typical growth culture characters recognised as being diagnostic for the species.

7. Phomopsis abietina (Hart.) Wilson et Hahn.

Lehrbuch d. Baumkr., II. auf., 1889, p.124;
 Bull.Soc.Mycol., VI. 1890, p.176; Trans.
 Brit.Mycol.Society, XIII, 1928, p.272.

Syn.: Phoma abietina Hart. (1889)

Dothierella pitya Prill.et Del., nec.Sacc.
 Bull.Soc.Mycol., VI. p.98 (1890).

Fusicoccum abietinum (Hart.) Prill. et Del.
 (1890).

nec.Phomopsis abietina (Hart.) Gröve, Jour.
 Bot.59.p.16 (1921)

HISTORY of the FUNGUS.

In his original description (1889) of the fungus associated with the classical Einschnürungskrankheit of the silver fir, (Abies pectinata) HARTIG (28) provisionally described the organism associated with the disease as Phoma abietina.

A year later PRILLIEUX & DELACROIX (46), in France, placed the fungus in the genus Fusicoccum, as F.abietinum (Hart.). They gave a full description of the fungus, emphasising the multilocular nature of the fruit body. Originally they had regarded this fungus as Dothierella pitya Sacc.(47) but upon a comparison with the type specimen of SACCARDO'S fungus (Fungi Veneti, Ser.IV., p.5; Sacc.Syll.Fung.III, p.241) decided they were dealing with quite another fungus.

In/

In 1921 GROVE (21) in an investigation of the fungus, made the new combination Phomopsis abietina (Hart.) describing the organism from specimens of Phomopsis on Douglas fir from Perthshire, Scotland.

The identity of Phoma abietina has been investigated, and the results of an intensive study of the species, have been recently published by WILSON and HAHN (77). The combination P.abietina Grove was apparently not based upon the fungus described by Hartig, and Prillieux and Delacroix; for Grove's description of the fungus differs considerably from that of the German and French authors. The fungus, is believed to occur only on the continent, and only on the silver fir host. For reasons just stated WILSON & HAHN made the new combination, Phomopsis abietina (Hart), which had a distinct significance from that used by Grove, and in doing so, referred only to the fungus described by Hartig, and Prillieux and Delacroix.

It is indeed interesting to know that typical Phomopsis abietina has not been recorded definitely outside of Germany and France. The fungus and the characteristic symptomatology of the disease with which the organism is associated, has not been discovered/

discovered in Great Britain or Scandinavia, where it has been searched for by the writer, STEVENSON (64.p.3.) reported the fungus from the U.S. on Abies Veitchii in Ohio, but an examination by the author of this specimen, showed that the organism was P. occulta. DOIDGE (16) reported P.abietina on Pinus sp. and Cupressus sp. from South Africa. An examination has not been made of these specimens. The organism on Cupressus would suggest either P. occulta, or possibly P.juniperovora.

DESCRIPTION of the FUNGUS.

The description of Phomopsis abietina (Hart.) Wilson et Hahn recently published (77) is repeated here in somewhat greater detail.

Phomopsis abietina (Hart.) Wilson et Hahn.

Pycnidia ectostromatic without a circumscribing dark line, scattered or aggregate, simple or compound, arising within and seated upon the cells of the upper cortical tissue of the host, amongst whose cells the fruit body is more or less incorporated; partially erumpent; black, carbonaceous, cone-shaped, lenticular, truncate or sub-globose; with or without an ostiole, inner pycnidium of simple fruit body formed from a single primordium, - unilocular (Pl.XX, Figs.1), or in larger primordia small/

small cavities forming in more than one plane, becoming multilocular, in which case there may be a fusion between chambers, so that an irregular unilocular cavity, a convoluted hymenium is formed, dehiscing at one ostiole, or the cavities may not fuse, in which case the fruit body remains multilocular, fuliginous, pseudoparenchymatous tissue overlying the cavity not heavily developed, variable in thickness; compound pycnidia (Pl. XX, Fig. 2) formed by the fusion of several primordia, exceedingly multilocular dehiscing at several ostioles, .08-.5 x .05-.4 mm; Spores of one type only, A type, hyaline unicellular, typically fusoid; elliptic (spindle-shaped) with acute or sub-acute extremities (Pl. XVIII, Fig. 1), symmetrical and asymmetrical, spores infrequently irregular, with protuberances (Pl. XVIII, Fig. 3), extreme range (100), 8.4-14.9 x 3.4-5.6 μ , commonly, (100), 10.9-14.0 x 4.0-5.3 μ , with one, generally two, occasionally three oil drops; sporophores, stout, subulate, persistent with tapering, acute, or subacute extremities, (Pl. XVIII, Fig. 2) Extreme range 6.7-13 x 1.0-3.4 μ , Spores exuded in a whitish tendril, or globular mass.

The perfect stage is unknown.

Hab. To be found only on the smaller branches of Abies pectinata on the continent in Germany and France. The fungus has not been discovered in Great Britain or Scandinavia.

EXAMINATION of EXSOCCATI.

- Specimen -Phoma abietina Hart. on Abies pectinata
coll. Hartig. (Hart.Path,Coll., Royal
Botanic Garden, Edinburgh)
- " -P.abietina Hart., on A. pectinata
coll. Hartig, Bavarian forest, (Herb.Tu-
beuf, Munich.)
- " -P.abietina Hart., ^{on} A. Pectinata, coll.Hartig
(Herb.Magnus, Hamburg.)

SPORE SIZE of PHOMOPSIS ABIETINA.

Variation of Spores in Nature.

In his study of Phomopsis abietina the author was unable to examine either of the type specimens from which Hartig, and Prillieux and Delacroix had made their original descriptions. However, an examination of the exsiccati of P. abietina just referred to, showed close agreement between these specimens with respect to spore size. The spore range of each of these three collections was within that determined for the species from freshly collected material from the Jura, France. Without a doubt the author was dealing with the species which Hartig intended. WILSON (76) had already commented upon the close agreement in size between the Edinburgh specimen and the original description.

VARIATION OF SPORES IN CULTURE.

A measurement study of spores produced on natural media in culture by Phomopsis abietina, form No. 43966, obtained from fresh specimens of diseased Abies pectinata, France, showed a range in length somewhat greater than that observed in nature. This difference in length of artificially produced spores is brought out in (TABLE XXXI). It will be observed that such spores were produced in the upper part of the range determined for naturally produced spores. The writer is inclined to the opinion, as a result of his experience with other of the Phomopsis forms studied in culture, to regard this fluctuation in size as not being directly due to the influence of the substratum.

Further investigation into the size of spores produced in culture will probably demonstrate that spores longer than these measured from specimen No. 43966 will be found, in which instance, the variation in size of spores in culture, can not be interpreted in terms of influence of the host substratum, but rather in terms of the inherent variability of the form within a specific range.

(TABLE/

(TABLE XXXI) COMPARISON: A. SPORES of P. ABIEETINA, NO. 43966 in NATURE and in CULTURE.

SUBSTRATUM	FUNGUS NO.	NO. SPORES	AVERAGE LENGTH X WIDTH, μ .	EXTREME RANGE LENGTH X WIDTH, μ .
<u>Abies pectinata</u> (Nature)	43966	70	12.4 x 4.6	8.4-14.9 x 3.7-5.6
Oat agar	43966B ⁵	10	11.5 x 3.8	10.2-14.6 x 3.7-4.3
<u>Pseudotsuga Douglasii</u> , twig	" B ⁷	10	13.8 x 4.6	11.2-15.8 x 4.3-5.3
<u>Ulmus campestris</u> , twig	" C ⁴	10	14.4 x 4.7	12.7-16.1 x 4.0-5.6
<u>Abies pectinata</u> , twig	" C ⁴	20	14.0 x 4.7	10.5-16.4 x 3.7-5.3
<u>Acer Pseudoplatanus</u> , twig	" C ⁴	10	13.6 x 4.6	11.5-15.5 x 3.7-5.0
Total. Culture	(5 Cult.)	60	13.5 x 4.5	10.2-16.4 x 3.7-5.6

SHAPE OF SPORES IN NATURE AND CULTURE.

When Hartig, and Prillieux and Delacroix described their organism Phoma abietina and Fusicoccum abietinum respectively, they did not mention the occurrence of irregularly-shaped spores amongst the typically spindle-shaped type which they observed. WILSON (76) however in differentiating his fungus, Phomopsis Pseudotsugae from Phoma abietina, described and figured the latter fungus after an examination of the Hartig specimen at the Royal Botanic Garden, Edinburgh. Wilson recorded an irregularity in form of the fusoid type.

The writer has examined the same fungus which Wilson reported. Unfortunately this specimen contained only a very scant number of spores. Those that were obtainable for measurement were decidedly irregular in shape. Further examination of other exsiccati, and living material of freshly collected spores in nature, showed that the regular spindle-shaped spore reported by the German and French investigators predominated, but that irregularly shaped spores such as those figured by Wilson, did/

did occur occasionally amongst the regularly shaped ones. The irregular spores were observed to appear only infrequently in the freshly collected material from the Jura.

In culture the typical fusoid shaped predominated; in certain of the cultures, however, the irregularly shaped spores were moderately abundant.

CULTURAL/

CULTURAL LIFE HISTORY STUDY OF P. ABIETINA.

The perfect stage of Phomopsis abietina has been variously reported upon. HARTIG (28) attempted to connect the fungus with the ascomycete, Peziza calycina Schum, but his experiments failed. REHM (49) gave Dasyscypha caliciformis Willd. as the perfect stage of this imperfect, but SCHELLENBERG (58) maintained the fungus belonged to another Discomycete. The French mycologists, including Prillieux, Delacroix and Henry, were not able to discover the ascigerous stage of the organism. At the present time the perfect stage of Phomopsis abietina is still unknown.

The writer attempted to secure the perfect stage of the silver fir organism by a growth of the fungus on both hard agars and on natural media, the latter placed at freezing temperature for a period of three months. Twigs of the following were used:- Abies pectinata, Pseudotsuga Douglasii, Alnus, Ulmus, and Acer. The perfect stage was not observed to form.

CULTURE/

CULTURE GROWTH CHARACTERISTICS.

Phomopsis abietina freshly isolated from Abies pectinata grew very slowly on artificial agars. On sugar-corn meal and Leonian's agars the colonies showed at the outset evidences of staling; this growth character became very pronounced after the second and third subculturing. On oat agar, however, growth while slow was not accompanied by staling. Upon this later medium a scant amount of fruiting was obtained, as well upon twigs of natural media. The aerial development of hyphae on twigs was very much reduced.

On Sugar-corn meal agar. Growth was exceedingly slow. The aerial hyphae were short, dense, felt-like, grayish, overlying a mid-stratum in which olivaceous colouring developed about the inoculum; later this green colour became brownish (old olive brown) tinging the aerial hyphae first at the periphery of the colony, which had staled pronouncedly, and then throughout the entire aerial growth. The colony after a month's growth attained a diameter of 4-6mm. Strands of hyphae were observed to grow deep into the/

the medium. These became dark brownish as did also the medium itself. Fertile pycnidia were not obtained on this medium.

On Leonian's agar. Practically the same culture growth characteristics were obtained on Leonian's agar as upon sugar-corn meal agar. On this medium staling was also very evident. Fertile pycnidia were not obtained. (Pl. XXIV, Fig. 1).

On oat agar. Growth on oat agar was slow but staling did not occur. The aerial hyphae at first was grayish, overlying a mid-stratum in which olive-green colour developed. With age this green colour became brownish. Fertile pycnidia were obtained, which were simple or compound and variously shaped. Neither on this medium, nor on the other two tested, did crystals form, such as were recorded for Phomopsis Boycei.

8. PHOMOPSIS BOYCEI s. sp.HISTORY OF THE FUNGUS.

In 1926, Dr. J.S. Boyce sent the writer interesting specimens of diseased branches of Alpine fir (Abies grandis, Lindl.) which he had collected in the vicinity of Clarkia, Idaho, U.S.A. He reported that the dead branches were associated with a fungus (no.40373) which macroscopically resembled a Phoma or a Phomopsis, and suggested that the organism might be Phomopsis (Phoma) abietina, which is commonly reported from continental Europe as killing branches of the silver fir. The attributed Idaho parasite, was observed to kill many branches.

The symptomatology of the Idaho canker on Alpine fir was identical with the well known "Einschnürungskrankheit" of the silver fir described and figured by HARTIG (29). The Phomopsis sp. according to Boyce, affected only the smaller branches, usually those not exceeding one half inch in thickness. He never observed it on the main stem, except occasionally killing the leader of small trees. In this regard it is interesting to note that HARTIG (29) and MER (40, 41) described a similar occurrence of Phoma abietina only on small branches of silver fir.

A critical examination of the fungus on Abies grandis showed it to belong to the Phomopsis group discussed in this paper, and to be closely akin to Hartig's Phoma abietina. Difficulty was experienced/

/Lowland

/Lowland or great

experienced at first in obtaining the Idaho organism in culture; for spores from material collected August, 1926, refused to germinate in ordinary tap water, Douglas fir leaves infusion, or on the surface of a sugar-corn meal agar plate. From a second collection (no.43977) of material made at Clarkia by Mr H.G. Lachmund during December of that same year, spores were secured which germinated readily. Isolations were obtained from monopycnidiospores, and from the advancing edge of a colony derived from a spore tendril planting. The culture growth characteristics of the isolated strains were in agreement. They also agreed with a culture which finally had been procured from the earlier collection (no.40373) by culturing hyphae of a mycelium tuft that had grown out of one of the cankers in moist chamber.

The spores in culture formed slowly, not appearing until 50 days after the culture had been made. These agreed essentially in shape and size with spores examined in nature, which were very distinctive in form. Naturally produced spores were extremely irregular, their tendency to become fusoid being almost completely disguised by protuberances which gave the spore bodies a decidedly angular appearance.

The literature has been searched for descriptions or figures of spores belonging to species with pycnidiospores similar to those produced by the Idaho *Phomopsis*/

Phomopsis. None were found until Mr E.W. Mason of the Imperial Bureau of Mycology, called the writer's attention to the spores of Vanderystiella Leopoldvilleana, P. Henn. (74) one of the Melanconiaceae, which were described and figured as being "fusoidetetradicis, acutis".

In the preceding section, spores with protuberances were discussed as occurring infrequently with the species Phomopsis abietina. In the case of the Idaho Phomopsis, the angular spore type was predominant, and not the regular fusoid form, as in P.abietina. Moreover, the culture growth characteristics were so very distinct for the two forms, that they could in no wise be confused (Pl. XXIV, Figs. 1&2). The Idaho fungus is therefore regarded as not only morphologically, but physiologically, distinct from the European species. It is therefore being described provisionally as a new species, and it is proposed to call it, Phomopsis Boycei, in honour of its discoverer, Dr. J.S. Boyce of the Forest Service, United States Department of Agriculture.

DESCRIPTION OF THE SPECIES.Phomopsis Boycei n.sp.

Pycnidia ectostromatic, scattered or aggregate, simple or compound, arising within and seated upon the cells of the cortical host tissue, amongst whose cells, the fruit body is more or less incorporated; partially erumpent; black, carbonaceous, cone shaped or sub-globose, breaking open by an irregular orifice; inner pycnidium of simple fruit body derived from a single primordium, - unilocular, cavity subspherical or elliptical with a superimposed layer which is not over thick, of dark fuliginous pseudoparenchymatous tissue, larger primordia become multilocellate, the chambers fusing irregularly and tending to form a unilocular cavity lined with a convoluted hymenium; compound primordia, distinctly multilocular; .01-.5 x .01 x .4 mm. (Pl. XX, Fig. 3); spores of one type: A type, hyaline, unicellular, fusoid becoming distinctly irregular with salient angles so that the spore becomes three or four sided, rarely symmetrical, elliptic-fusoid, with acute or subacute extremities; extreme range (100) 9.0-13.0 x 4.0-7.8 μ , commonly (100) 9.9-12.4 x 5.0-6.8 μ , average 11.1 x 5.9 μ , (these measurements are very close to those calculated by Dr. J.S. Boyce, extreme range (50), 9.6-13.7 x 5.8-8.9 μ ; sextile range, (50), 10.3-12.7 x 6.2-6.9 μ ; average (50) 11.5 x 6.7 μ): with one or two large guttules (Pl. XIX, Fig. 1); sporophores, short, subulate, with tapering extremities, 5.0/

5.0-9.9 x 1.0-2.5 μ ; (Pl. XIX, Fig. 3); spores exuded in a whitish tendril or droplet; several tendrils emerging from a compound fruit body.⁽¹⁾

Perfect stage unknown.

Hab. Type, no. 40373 coll. J.S. Boyce on Abies grandis, Clarkia, Shoshone Co., Idaho, U.S.A.

August 31, 1926. Also collected by H.G. Lachmund, on A. grandis, Clarkia, Ida., Dec. 1926; by Boyce, on A. grandis, Santa, Shoshone Co., Ida., June 25, 1927; by C.R. Stillinger, on A. grandis, Libby, Mont., August 26, 1927; by Stillinger, on A. grandis, Hougau, Mont. August, 31, 1927; by Lachmund, on A. grandis, Coeur d'Alene, Kootenai Co., Ida., September 9, 1927.

(1).
Phomopsis Boycei, n. sp.

Pycnidiis discretis vel aggregatis; sub initio subepidermicis denique erumpentibus, carbonaceis, lenticularibus, conoideis, truncatis, vel subglobosis, basibus complanatis, ostioli paulo pertusis, vel deficientibus, contextu heterogeneo praeditis, matricibus fuligineis supra cavitates sporiferas incrassatulis; pycnidiis parvis, majoribus multilocularibus vel per dissepimentum disruptionem unilocularibus, ceteris unilocularibus, .01-.5 x .01-.4mm. sporulis, hyalinis, continuis, egguttulatis, inaequalis, fusoidetriedricis vel fusoidetetradricis, acutis, rarus ellipticis, (100) 9.0-13.0 x 4.0-7.8 μ , vulgo, (100) 9.9-12.4 x 5.0-6.8 μ ; basidiis continuis, subulatis, brevis, tenuis, 5.0-9.9 x 1.0-2.5 μ .

Hab. in ramis emortuis Abietis grandis, Lindl. in America boreali (Idaho et Montana). August, 1926.

SIZE OF SPORES OF PHOMOPSIS BOYCEI
IN NATURE AND CULTURE.

A measurement study of the average size and range of three collections of Phomopsis Boycei on Abies grandis; indicated a range practically identical for the three forms, with slight variation of the average within this range (9.0-13.0 x 4.0-7.8 μ).

In culture Phomopsis Boycei produced spores on oat agar and natural media, whose average (50 spores) showed satisfactory agreement with that of spores produced in nature, and whose range with regard to length was slightly greater (1 μ). When compared however with the measurements contributed by Boyce (see species description), this extension of the upper limits of the range in culture, agreed rather closely with the figures given by Boyce for the form which he measured.

(TABLE XXXII.) BIOMETRICAL CONSTANTS of P. BOYCEI and P. ABIETTINA COMPARED.

POPULATION	NO. SPORES	M	LENGTH		STANDARD DEVIATION	COEFFICIENT of VARIABILITY
			SIGNIF. of MEAN DIFFERENCES	SIGNIF. from		
<u>P. Boycei</u>	100	11.2 ±.061			.897 ±.043	8.61 ±.385
<u>P. Abietina</u>	100	12.3 ±.085	Signif. from		1.252 ±.060	10.18 ±.491
			WIDTH			
<u>P. Boycei</u>	100	5.9 ±.054			.793 ±.038	13.43 ±.652
<u>P. Abietina</u>	100	4.5 ±.034	Signif. from		.508 ±.024	11.28 ±.545

To determine a probable significant difference between spore populations of Phomopsis abietina and P. Boycei, respectively, 100 spores each of the two species were treated biometrically. A significant difference was found which is presented in Table XXXII.

VARIATION IN SHAPE OF SPORES OF P. BOYCEI
in NATURE AND IN CULTURE.

As already has been stated the spores of Phomopsis Boycei were readily recognised by their peculiar irregularity of shape. The spore population contained a scant number of the symmetrical, elliptic type with subacute extremities. It is this type that Hartig, and Prillieux and Delacroix described as characteristic for Phoma abietina(Fusicocum abietinum). For the most part the spores of P.Boycei were exceedingly irregular, with protuberances so that they appeared angular with salient angles; often they became much distorted (Pl. XIX, Fig. 1).

Spores of Phomopsis Boycei produced in culture were likewise very irregular in shape; the protuberances were also very angular.

CULTURAL LIFE HISTORY STUDY OF P. BOYCEI.

The perfect stage of P. Boycei is unknown. Cold storage experiments wherein twigs of Ulmus, Acer, Alnus, Pseudotsuga and Abies inoculated with monopycnidiospore cultures, and with cultures obtained from a spore tendril planting were placed at approximately freezing temperature for three months produced only the imperfect stage, when these tubes were brought back to room temperature.

CULTURE GROWTH CHARACTERISTICS.

Phomopsis Boycei was cultured for approximately two years. It maintained a vigour of growth and continued to produce culture growth characteristics which are herein reported as characteristic for the species. Pycnidia also continued to form, producing the very irregular shaped spores. One culture characteristic, which this species possessed along with P. juniperovora, was the ability to stimulate the formation of crystals in the medium. The crystals were yellowish, and formed deep within the agar slant. They continued to form in cultures after a period of two years sub-culturing.

On Sugar-Corn Meal Agar. Growth at first was slow, appressed, colourless. The aerial growth was whitish, minutely granular, becoming coarsely granular, overlying a midstratum in which a trace of dull olivaceous colour developed. Within 2 to 3 weeks yellowish crystals developed in the medium, and tinges of sulphur yellow appeared in the now grayish aerial stratum.

Growth upon Leonian's agar was very similar to that on sugar-corn meal agar. On the former both the crystals and the sulphur yellow colour developed; fruiting was very poor. Better pycnidial production was obtained on the sugar-corn meal medium. (Pl.

XXIV, Fig. 1).

PATHOGENICITY of CONIFER PHOMOPSES.

It has already been indicated upon the basis of previous investigations, that Phomopsis juniperovora has been shown by Hahn(22,24) to be a parasite of nursery and ornamental stock of the Cupressaceae in the U.S. He also demonstrated artificially the parasitism of this organism (24) on Douglas fir and European larch.

Bottomley(3) in South Africa proved the parasitism of a Phomopsis sp. on nursery stock of Cupressus. This organism was provisionally regarded as Phomopsis juniperovora, although the actual identity of the South African fungus, with the parasite in the U.S. has not been satisfactorily shown.

Wilson(76) described the Phomopsis disease of the Douglas fir and other conifers in the British Isles, to which he ^{signed} attributed Phomopsis pseudotsugae as the probable cause. Artificial inoculation experiments with this organism have been performed by him and the author, the results of which will be published shortly.

The writer has conducted a limited number of artificial inoculation experiments with the following Phomopsis spp., - P.conorum, P.occulta, P.abietina. The experiments, in which only negative results were obtained, will be briefly presented:

Inoculations/

Inoculations with *Phomopsis conorum*.

Phomopsis conorum, a fungus which Rostrup (52; 25) confused with Phoma pitya, had been attributed and figured by him as the cause of a die-back of Douglas fir and other conifers. Amongst his earlier collections of P. Pseudotsugae, the writer frequently discovered and isolated the organism from frosted nursery stock of Douglas fir and spruce. He has never observed the fungus fruiting on a typical body canker of the Douglas fir, such as has been figured and described by Wilson (76) as caused by P. Pseudotsugae.

May 12, 1926. Fifteen 7-year old potted trees of Douglas fir (Pseudotsuga Douglasii) were inoculated upon the main stem and small lateral branches, -45 inoc.; controls, 19. Bits of sugar-corn meal culture, forms, no. 43917, -22, -27, were inserted beneath a small tongue slit wound, which had been made with a sterile scalpel, to the cambium; the inoculation was tied down with a bit of cotton wool moistened with sterile water; controls were treated likewise except that the fungus culture was omitted. (1) Negative results.

May 13, 1926. Four 3-year old potted trees of Larix europea were inoculated upon the main stem and laterals, -18 inoc.; controls, 10. Culture form, no. 43928, was used. Negative results.

(1) Artificial inoculations in subsequent inoculations were made in a similar fashion. In each instance the tissue surface was sterilized at the outset with methyl spirits before wounds were made.

June 22, 1926. Three 15-year old plantation trees of P. Douglasii were inoculated upon the main trunk (9 inoc.) and branches (38 inoc.); controls, main trunk, (3 inoc.) and branches, (14 inoc.) with form, no. 43927.
 Total, -47 inoc.; 17 controls. Negative.

July 7, 1926. One 15-year old tree of P. Douglasii was inoculated, 8 inoc., in 1yr., 2yr., 3yr., old wood at the point of infection; 3 controls; with form no. 43927. Negative results.

Inoculations with Phomopsis occulta.

The writer has published (24, p. 905) positive results in inoculation experiments with Phomopsis forms now known to be forms of P. occulta (no. 41037, from Taxus; no. 41040, from Taxodium; no. 41039, -45, from Pseudotsuga taxifolia (Poir.) Britt. (P. Douglasii).

In the United States Phomopsis occulta has been observed associated with damage among ornamental plantings of conifers, which were growing in situations not conducive to vigorous healthy growth, or plants were infected which were known to have been injured by other environmental causes; in which instances the Phomopsis could only be considered secondary.

Phomopsis occulta has been observed frequently on frosted nursery stock of Douglas fir in Great Britain. The perfect stage of this fungus is to be found commonly on suppressed branches, and upon the ground litter of prunings in Douglas fir plantations.

Oct. 15, 1926. One 15-year old tree of P. Douglasii was inoculated upon the main trunk(2 inoc.), and upon the branches(10 inoc.) in 2 and 3 year old wood at the point of infection. controls(5); with form no.43957.
Total 12 inoc.; negative results.

Inoculations with *Phomopsis abietina*.

"Bohm(2) reported negative inoculation results with Fusicoccum abietinum(Phoma abietina) upon Pseudotsuga Douglasii in 1896. At that time he drew attention to the fact that previously this fungus was only known on the silver fir. The organism investigated by him was described as producing spores which were "extraordinarily small, pointed at both ends and with one or more oil drops". Böhm further commented, "There are now so large a number of Phoma-forms, that each plant species may be considered as having a different form. The differences between these forms are many times so slight that a correct determination is not an easy matter. I am greatly indebted to Prof. Magnus in Berlin; for he has looked at my preparations and verified my determinations as correct." Inasmuch as Phomopsis abietina according to our present knowledge does not occur on the Douglas fir, in all probability Böhm was dealing with the fungus P.conorum.

Böhm called attention to a similar disease of the Douglas fir in France which had been investigated by/

Mer(41) in 1893. Mer(40;41) had discussed in great detail the ["]Einschnurungskrankheit of the silver fir. He(41) attempted artificial infections presumably with Hartig's Phoma abietina but failed in obtaining positive results.

Curiously enough, Phomopsis abietina does not infect the trunk or larger branches of the silver fir, but attacks only those branches about a finger's thickness, or smaller, upon which it causes a typical constriction. In this respect it resembles P. Boycei upon Abies grandis in north western North America.

Nov. 19, 1926. Seven potted 6-year old trees of Abies pectinata were inoculated with a freshly isolated culture strain, of P. abietina, no. 43965A¹, 2 inoc. to a tree in the 3 and 4 year old wood at the point of infection; controls, (1) on each tree, in the 5 year wood just below.
Total 14 inoc.; negative results.
(7 controls)

May 23, 1928. Seven potted trees used in the experiment of Nov. 19, 1926, were reinoculated, with single spore culture strain no. 43966D; 2 inoc. to a tree in the 2 and 3 year old wood at the point of infection; controls (1) on each tree below in the 4 year old wood.
Total 14 inoc.; 7 controls.
Negative results.

Conclusions/

Conclusions.

The negative artificial inoculation results obtained in experiments with Phomopsis occulta, P. con-
orum, and P. abietina, indicate two possible conditions (1) that the writer had not hit upon the proper environmental conditions in which these organisms naturally become active parasitically, or (2), that the organisms in themselves are only to be considered weak parasites, which are unable to attack the plant until it has been primarily injured by some other environmental factor which has reduced its vitality and resistance; in this event the Phomopsis species become induced successional parasites.

DISCUSSION.

From the evidence brought forth in this paper, it would seem that the genus Phomopsis on the basis of investigational work with the eight species on conifers dealt with, showed generally a marked constancy with regard to the size and shape of the A and the B spores, both in nature and in culture. Variation in size occurred, but seemingly within a specific size range determined for each group of forms. It was found that this size range, representative of the species, could be estimated best by a measurement study of the species throughout its host range. A correlation of this morphological evidence with culture growth characteristics, gave further proof that the same physiological forms were being considered. Throughout the investigation of Diaporthe conorum it was observed also, that the ascospore size range was specific, and that variation occurred within a given range. With respect to the constancy of ascospore size it is here interesting to note that SHEAR, STEVENS, and WILCOX (61) reported both species, Botrysphaeria ribes, G. et D. and Physalospora malorum, (Berk.) Shear, as showing remarkable uniformity in the size of their ascospores throughout their host range, whether produced in culture or on the host. Cultural growth characteristics of forms from different hosts were uniform for each species. On the other hand they did not report the/

the same uniformity of size of pycnidiospores of the above species from different hosts. They further stated that as yet they had no satisfactory information as to the extent to which the size of the pycnidiospores could be modified by the environment. In a paper of the year previous STEVENS and JENKINS (63) were of the opinion, however, that pycnidiospores and ascospores of Botrysphaeria ribis were in satisfactory agreement with regard to size, despite the fact that they came from widely separated hosts (Rose, Aesculus hippocastanum, and Ribes).

SHERBAKOFF (62) in his monographic treatment of the genus Fusarium on potatoes stated that the most important character in the classification of this group was the type and shape of the conidia, which was sufficiently stable to be used safely in a morphological treatment of this difficult group. Even the size of conidia he found, showed a surprising uniformity and stability, when conidia of the same type were compared. WEHMEYER (69) in his study of the genus Diaporthe regarded spore size as being chiefly valuable as a specific character.

There has been considerable controversy concerning spore size and the influence of the substratum on that particular character. That the size of the individual spore is not fixed was brought out in an interesting experimental study by HANNA (26) who attempted to produce large spored strains and small spored/

spored strains in pure lines of Coprinus sterquilinus, Buller, by the continuous selection of large and small spores. He was unable to find satisfactory evidence of the inheritance of individual variations in spore size. Hanna suggested that inasmuch as the mean size of the spores of different fruit bodies of one and the same species of Hymenomycete, vary considerably, the spore size given by systematists for determining Hymenomycetous species ought to be based on measurements of the spores of a number of fruit bodies obtained in different places.

Almost generally wherever ascospore or pycnospore size in nature and culture have been investigated, workers in the genera Diaporthe and Phomopsis (27; 71, p.393; 79) have reported agreement. When one considers for example the excellent uniformity of spores of P. occulta, from a number of widely separated conifer hosts, one must admit a certain constancy in spore size so far as the range is concerned for that genus, whether the spores be produced in nature or in culture. This character of size, along with that of spore type and shape, offered the best basis for species distinction amongst the forms of Phomopsis on conifers.

The present conception of variation in size within a specific range has been emphasised by WEHMEYER (71, p. 377) in other species investigated within the groups. In the case of Diaporthe pruni, ~~EetE~~ he investigated thirty-two species described on Prunus and/

and related host genera. All these species he found to have practically the same stromatic configuration, but to show slightly different ranges of ascospore and conidial measurements. As a result of his researches among this comparatively large number of forms among which an overlapping variation on related host substrata occurred, he came to the conclusion that these so called species, were synonymous. When one considers how little, if any, the size of ascospores and pycnidiospores of Diaporthe conorum were affected when produced on natural media of broad-leaved hosts, e.g., Ulmus campestris, one begins to realise the specific nature of the spore range at least for this group. For this reason the relationship between certain conifer Diaporthes and Phomopses, with forms on broad-leaved hosts is very strongly suggested.

The author was unable to obtain germination in a limited number of tests of the B spores which are borne on true sporophores in exactly similar fashion to the spores of the A type. In so doing he repeated the experience of a large number of workers who also attempted the germination of this enigmatic spore body and failed. ROBERTS (50), HARTER and FIELD (27), FAWCETT (18), BUTLER and KHAN (8), CAYLEY (9), MARCHAL (38), WINSTON, BOWMAN, and BACH (79), BROOKS (6), WILSON (78), have all reported negative results in germination studies of B spores.

ARCHER/

ARCHER (1, p. 18) on the other hand reported the ready germination of the filiform spores of Phomopsis Arctii and in doing so, called attention to the fact that the positive results obtained by him were of great interest because it was rarely that any one succeeded in germinating the B spores. Archer drew attention to BREFELD'S (5, p. 37) results in germinating the filiform type in several species. Unfortunately according to Brefeld's description of the germination of the filamentous spores of Aglaospora profusa, his results were inconclusive. After various attempts with negative results that investigator was able finally to get a general germination of the B spores. He stated, "at favourable places germ tubes appeared, which were very much swollen and became two times as thick as the spore. But their growth proceeded so slowly, that the culture was contaminated by foreign fungi, when the germ tubes had reached about the length of the spores." In the case of Diaporthe tenuirostris, Nke., filamentous spores suddenly produced knotty swellings after they had been placed in a nutritive solution, without producing a germ tube, whereas Diaporthe rudis, (Fr.) Nke., and D. Spina, Fkl., and D. inaequalis, (Curr.) Nke., produced one or two outgrowths which lengthened slowly and remained very thin.

Brefeld's results do not appear very convincing. The author is inclined to regard such phenomena/

phenomena as described by him in line with his own results with B spores of Diaporthe conorum (see p.134). As in the case of CAYLEY (9), and of WILSON (78) who investigated the germination of the B spores of Myxosporium, the writer obtained lateral processes or swellings at the tips of the B spores which extended only for an extremely short length, and then further growth terminated. FAWCETT (18p.110) described a curious bulging of the protoplasm from the end of hyphal branches when Phomopsis citri was grown in either dilute prune or orange juice. He stated that distinct balls formed giving the impression of spore formation. It is difficult to find a explanation for such phenomena except it be upon a physical basis.

Unfortunately ARCHER (1) did not figure or give in detail the data obtained in his germination studies of Phomopsis Arctii . In an endeavour to repeat his results the author applied to the Centraal-bureau voor Schimmelcultures, Baarn, Holland and received a culture of P. Arctii which had been deposited there by Archer. Unfortunately, this culture was sterile. Efforts to bring it back to a fruiting condition were negative.

In this respect it is interesting to note here that the writer at one time considered that he had germinated the B spores of Phomopsis occulta; for a dilution of spores from what appeared to be a tendril composed/

composed of B spores solely, made in a hanging drop of sterile distilled water produced here and there a germ tube. These tubes however, when traced back to their source, were found to originate from minute abnormally small A spores and not from the B spores.

POTEBNIA (45) figured and described germination of microconidia ^{of} Phacidiella discolor, (Mout. et Sacc.) A.Pot.. In this instance a typical mycelium was obtained. There is apparently no doubt about Potebnia's results. At the same time the question comes up as to whether the microconidia of Phacidiella are to be considered in the same light as the filamentous B spores of a Phomopsis. Cytological evidence can only decide this.

CAYLEY (9, p. 261) investigated the nucleus of the B spores of Diaporthe pernicioso but in stained preparations could not find a definite nucleus; the cell contents were more or less granular and stained deeply. At an earlier date ENLOWS (17) described the B spores of Phomopsis Kalmiae as containing several oil drops, and very rarely a very pale staining nucleus. Further cytological evidence may throw light upon the nature of these much discussed spore bodies and their function in the life-histories of the fungi to which they belong.

In connection with the controversy regarding the germination of these spores, the discussion as to the time of their first appearance has come up.

BREFELD/

BREFELD (5) contended that they came after the A type had formed; other investigators (1, 9, 27, 67, 72, p. 166, 170) maintained they were formed first, and the A type followed. BUTLER and KHAN (8) stated that in the case of Hendersonina sacchari, Butl. the A and B spores appeared to arise simultaneously.

Investigational work with the Phomopsis species on conifers indicated that the last two types of A and B spore formation occurred. The following observations seem to hold: (1) that where culture growth conditions were unfavourable for A spore production, large sterile stromata frequently formed or an occasional stroma in which B spores were found; (2) that abnormal cultures gave rise to the production of small pycnidia bearing mostly B spores; (3) that when mostly B spores were found, the A type occurring along with these, were abnormally small. Given favourable growth conditions forms producing abnormal A spores were found to be potentially fully capable of reproducing spores quite normal in size. In this respect it is interesting to note the opinion of WEHMEYER (66, p. 249) who observed the B spore type produced in the life-history study of Diaporthe oncostoma, as appearing "under favourable growth conditions." Contrasted with this opinion is that of MARCHAL (38) who observed microconidia as being more numerous on poor media and in small pycnidia. Whatever the particular factor or factors may be which are concerned in stimulating the formation of the B spores/

spores, the origin of these bodies can probably be explained on a physiological basis, and as ARCHER (1) suggested some knowledge may be gained of the determining factors in their formation by means of carefully controlled experiments with a large number of species of Phomopsis in culture.

In the culture life-history of Diaporthe conorum, no evidence was found of the necessity of the union of two sexual strains for the formation of perithecia. The perfect stage was obtained as readily in culture from monoascospore isolation cultures, as from monascus cultures, or cultures in which two monoascospore strains were mixed.

The perfect stage was also obtained from monopycnidiospore isolation cultures derived from spores produced under artificial conditions by monoascospore strains.

Only two strains of Phomopsis occulta out of 83 investigated, collected from a comparatively large variety of hosts, widely distributed geographically, produced the perfect stage. In this instance a monopycnidiospore isolation was not made, the culture being obtained from a spore tendril taken from a stroma in the lower parts of which the perithecial initials of Diaporthe were to be found. One culture obtained from mycelium isolated from the inner bark tissue likewise reproduced the perfect stage. All other strains of P. occulta reproduced themselves generation after generation both on hard agars and natural media, without/

without the Diaporthe stage appearing.

In all 177 strains of conifer Phomopses were investigated. In no case was the perfect stage observed except in the two instances just mentioned. Strains of P. Pseudotsugae were mixed in culture two strains to a tube, but such a mixture apparently had no effect either in stimulating perithecia or even an exceptional production of the imperfect fruiting bodies. WEHMEYER (67, 68, 69, 70, 71, 72) in his life-history investigations of the genus Diaporthe repeatedly demonstrated the cultural connection between this ascomycete and Phomopsis. He preferred to work from the perfect stage end of the cycle however, in proving cultural relationships. His cold temperature methods (67) were followed by the author in experiments for developing the ascogenous stage from the imperfect, but only negative results were obtained. CAYLEY (9) obtained large numbers of perithecia in monoascospore cultures on various media. Perithecia of D. perniciosa developed sparingly in cultures from pycnospores.

Within the genus Diaporthe it would appear that forms are probably in a state of flux and where at one time the Diaporthe stage formed a regular part of the life-history of the fungus, certain strains are now able to continue reproducing themselves quite independently without the ascomycetous stage. An isolation of sex is strongly indicated for the genus Diaporthe which appears to be no longer dependant upon the union of two sexual strains for its continuity.

SUMMARY.

An investigation of the present known Phomopsis species on conifers has been undertaken. Eight species are differentiated and their synonymy is given; two species are described provisionally as new, - Phomopsis Montanensis n. sp. and P. Boycei n. sp.

The cultural life-history of Diaporthe conorum, (Desm.) Niessl (syn: D. occulta, (Fuck.) Nke., D. pitya, Sacc.) is given. Monoascospore cultures of D. conorum produced both the perfect and the imperfect stage, Phomopsis occulta, Trav.. Monopycnidiospores obtained from a culture derived from a monoascospore isolation, reproduced the ascomycetous stage, D. conorum.

Diaporthe conorum produced the perfect stage as readily from monoascospore cultures, as from cultures derived from monoascus isolations, or the mixture of two monoascospore strains. The addition of Taka-diaxase to cultures of D. conorum did not appreciably stimulate perithecial production.

Phomopsis conorum, (Sacc.) Died. is a species distinct morphologically and physiologically from Phomopsis occulta (D. conorum). The perfect stage of P. conorum, which inhabits not only cones but other plant parts as well, is not known.

Limited tests in which strains of Phomopsis Pseudotsugae, Wilson from Great Britain and the continent, were mixed in culture, gave negative results so far as ascogenous stage formation was concerned. The/

The perfect stage of this fungus as yet has not been proven definitely.

Phomopsis species isolated from conifers showed a general agreement amongst their forms with regard to the constancy and persistency of the culture growth characteristics. This uniformity applied equally to forms collected from hosts widely separated both geographically and phylogenetically.

Spore shape proved to be a valuable morphological character for the differentiation of species. The shape of culturally-produced spores both A and B showed generally excellent agreement with that of spores produced in nature.

Variation in spore size occurred both for the ascospores of Diaporthe conorum and the pycnidiospores of the various Phomopsis species investigated. This variation took place within a given specific range which appeared to be fairly constant. It was found that the specific range of the fungus species could be determined by a study of forms of the particular organism throughout its host range.

Neither the shape nor the size of ascospores was influenced by artificial growth of D. conorum on broad-leaved host substrata. The relationship between this conifer Diaporthe, and forms on broad-leaved hosts is indicated.

The conifer Phomopses showed both wide and extremely limited host relationships. Certain of the species are now known to be widely distributed geographically/

geographically, occurring on a comparatively large number of hosts, e.g., P. occulta, occurred on 14 host genera and its habitat included both North America and Europe. Species such as P. abietina appeared to be limited to a single host, and to the smaller branches of that host. This fungus is known only to occur on the continent in Germany and France.

In artificial inoculation experiments, negative results were obtained in attempting to infect Abies pectinata with Phomopsis abietina, (Hart.) Wilson et Hahn.

Negative results are also reported for Phomopsis conorum upon Pseudotsuga Douglasii, and for P. occulta from the same host.

A discussion upon the formation and germination of the B or filamentous spore is given, together with a brief consideration of sex in the genus Diaporthe. There were evidences of sex isolation in the group Diaporthe; for strains of this ascomycete were fully capable of reproducing the perfect stage from monoascospore isolation cultures. Strains of Phomopsis occulta isolated from nature rarely produced the perfect stage. Only two cases were observed. Eighty-one forms of Phomopsis occulta continued to reproduce the imperfect stage generation after generation on both hard agars and natural media. In all 177 forms of conifer Phomopses (8 species) were observed in culture and the perfect stage was found only in two instances.

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DESCRIPTION OF PLATES.

All figures of ascospores, pycnidiospores,
and sporophores, are x 2000.

PLATE I.

- Fig. 1. Reproduction: Diaporthe pitya, Sacc.
Sacc. Fungi ital. no. 1277, 1882.

PLATE II.

- Fig. 1. Ascus, and ascospores, Diaporthe conorum,
(Desm.) Niessl (no. 43979, Sitka spruce).

PLATE III.

- Fig. 1. Shape of ascospores compared:-
Sphaeria conorum, Desm., type specimen.
Fig. 2. Sph. conorum, ex. 1773.
Fig. 3. Sph. conorum, ex. 913.
Fig. 4. Valsa occulta, Fuck., ex. 622.
Fig. 5. Diaporthe conorum (Desm.) Niessl, no. 43931,
Douglas fir form.
Fig. 6. D. conorum, no. 43979, Sitka spruce form.

PLATE IV.

- Fig. 1. Elongate, flexuous, perithecial beaks of
Diaporthe conorum, cone scale of Larix
europaea, Glentress, Peebles-shire, Apr. 1928.

Approx. 21 x natural size.

PLATE V.

- Fig. 1. Circumscribed stromata of D. conorum on
a cone scale of Picea excelsa; spore
tendrils of Phomopsis occulta, Trav. shown.
Kelso, Roxburghshire, May, 1928.

Approx. 15 x natural size.

PLATE VI./

PLATE VI.

- Fig. 1. Mass of perithecial beaks of Diaporthe conorum form no. 43979, Sitka spruce artificially produced on a sterile twig of Ulmus campestris within 3 months, from a mono-ascospore culture, 43979B^{VIII}.

Approx. 20 x natural size.

PLATE VII.

Phomopsis occulta, Trav.

- Fig. 1. A spores.
 Fig. 2. Intermediate spores.
 Fig. 3. B spores.
 Fig. 4. Sporophores.

PLATE VIII.

B spore germination study.

- Fig. 1. Processes produced by B spores of P. occulta, no. 43965, in malt extract infusion, 0.5%, at room temperature within 24 hours. These processes were not observed to lengthen further.
 Fig. 2. Longest process produced by a B spore.

PLATE IX.

Phomopsis juniperovora, Hahn.

- Fig. 1. A spores.
 Fig. 2. Intermediate spores.
 Fig. 3. B spores.
 Fig. 4. Sporophores.

PLATE X.

Phomopsis conorum, (Sacc.) Died.

- Fig. 1. A spores.
 Fig. 2. B spores.
 Fig. 3. Intermediate spores.
 Fig. 4. Sporophores, of A type spores.
 Fig. 5. Sporophores, of B type spores.

PLATE XI./

PLATE XI.

- Fig. 1. Polymorphic pycnidial stromata of Phomopsis conorum form no. 43928, Douglas fir, on a sterile Douglas fir twig in culture.

Approx. 20 x natural size.

PLATE XII.

A spores Phomopsis spp., compared:-

- Fig. 1. P. occulta; note the presence of occasional A spores pointed at one end.
- Fig. 2. P. juniperovora; note the "pinching in" at the median part of the spore.
- Fig. 3. P. conorum; note the typical boat shape with acute extremities.

x 1000.

PLATE XIII.

Stromatic pycnidia of Phomopsis occulta and P. conorum.

- Fig. 1. Stroma of P. occulta in the upper cortex of Douglas fir, circumscribed by a black line; in the stroma are embedded the lens shaped cavities formed in one plane, with a considerably thickened layer of pseudoparenchymatous tissue above.

x 80.

- Fig. 2. Pycnidial cavity of P. occulta on Douglas fir, with a thickened layer above.
- Fig. 3. Pycnidia of P. conorum on a cone scale of Picea excelsa, tending to fuse forming an elongate, irregular cavity in one plane with a thickened layer above. Note the two ostioles in the compound pycnidium where two primordia have fused.

x 45.

PLATE XIV.

Phomopsis Montanensis, n. sp.

- Fig. 1. A spores.
- Fig. 2. B spores.
- Fig. 3. Sporophores of A and B spores.
- Fig. 4. Sporophores of A spores.

PLATE XV./

PLATE XV.Phomopsis Strobi, Syd.

- Fig. 1. A spores.
 Fig. 2. Sporophores.
 Fig. 3. A spores (from culture isolated by Petri from Abies pectinata).
 Fig. 4. Sporophores, Petri culture.

PLATE XVI.Phomopsis Pseudotsugae, Wilson.

- Fig. 1. A spores.
 Fig. 2. Elongate A spores, occasionally occurring.
 Fig. 3. Sporophores.

PLATE XVII.Stromatic pycnidia of P.Montanensis & P.Pseudotsugae.

- Fig. 1. Pycnidium of P.Montanensis on Abies lasiocarpa showing the irregular hymenium and unilocular cavity formed, after the septa have broken down which were produced as the result of the earlier multilocular condition of the fruit body.

x 50.

- Fig. 2. Unilocular cavity of a pycnidium of P.Pseudotsugae formed as the result of the fusion of smaller cavities, formed in more than one plane.

x 80.

- Fig. 3. Compound multilocular fruit body of P.Pseudotsugae on Douglas fir; note the small pycnidium buried in the cortical tissues below.

x 130.

PLATE XVIII./

PLATE XVIII.

Phomopsis abietina (Hart.) Wilson et Hahn.

- Fig. 1. A spores.
 Fig. 2. Sporophores.
 Fig. 3. Spores with irregular protuberances, occasionally occurring.

PLATE XIX.

Phomopsis Boycei n. sp.

- Fig. 1. A spores.
 Fig. 2. Regular, elliptical A spores, occasionally occurring.
 Fig. 3. Sporophores.

PLATE XX.

Stromatic pycnidia of P.abietina and P.Boycei.

- Fig. 1. Simple and compound pycnidia of P.abietina on Abies pectinata.
 Fig. 2. Compound pycnidium of P.abietina.
 Fig. 3. Compound pycnidium of P.Boycei.

x 130.

PLATE XXI.

Cultures of forms of Phomopsis occulta on Leonian's agar compared. Forms isolated from Taxus, Sequoia, Thujopsis, Juniperus, Thuja, Larix, Abies and Pseudotsuga, in North America and Europe, are shown.

PLATE XXII.

Cultures of forms of Phomopsis occulta isolated from Douglas fir on Leonian's agar compared. Forms isolated from the United States, Canada, Scotland, England, and Wales are shown.

PLATE XXIII.

PLATE XXIII.

Cultures of forms of Phomopsis conorum isolated from Douglas fir on Leonian's agar, compared.

PLATE XXIV.

Cultures of four species of Phomopsis on Leonian's agar compared.

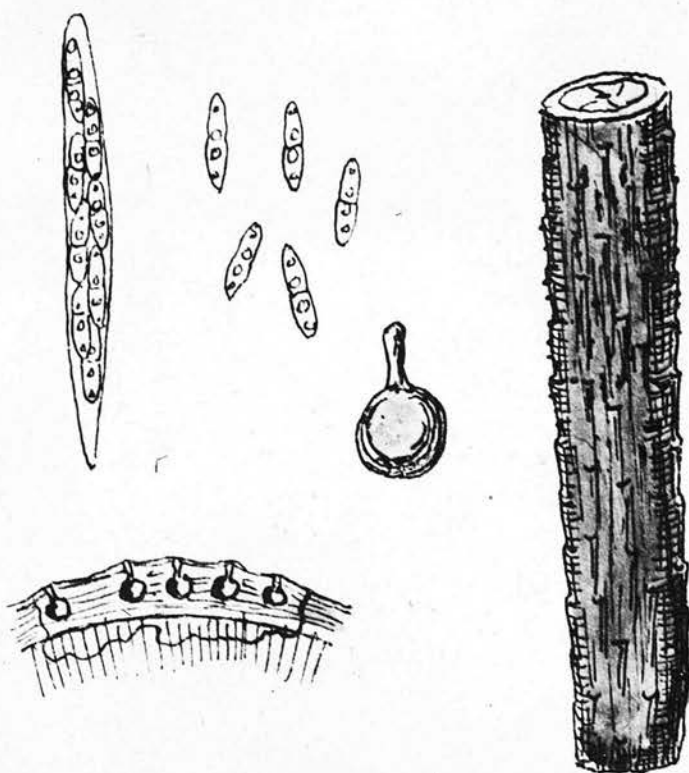
- Fig. 1. Phomopsis Boycei n. sp.
Fig. 2. Phomopsis abietina.
Fig. 3. Phomopsis Pseudotsugae.
Fig. 4. Phomopsis Strobi (from Petri, Italy).

Sacc. H. Ital.

Pyrenom.

asci 50-55x6

spor. 10-12x3,5-4.



Diaporthe (T.) pithya Sacc.—

Sacc. Syll. I, p. 689. —

In ramis Abietis excelsae, Selva (Treviso). Aut. 1873.

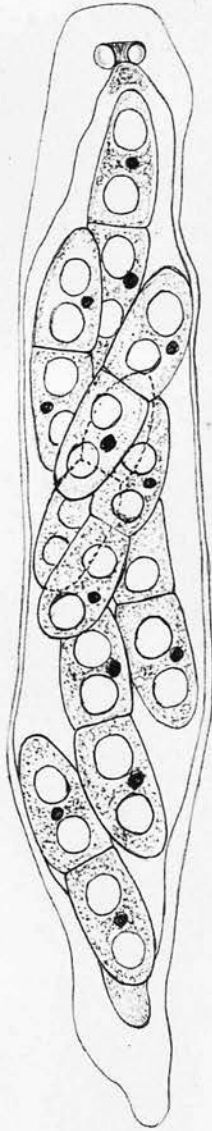


fig. 1.

Plate III

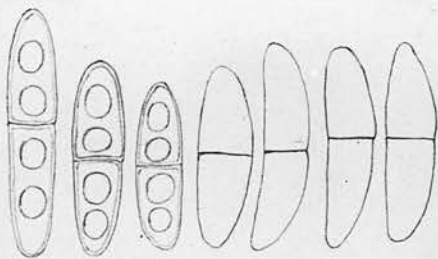


fig. 1.

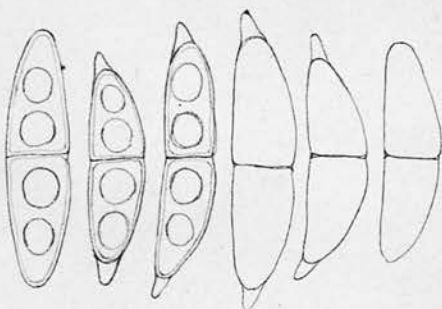


fig. 2.

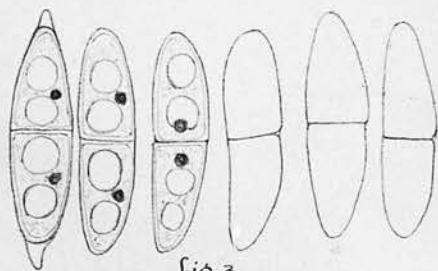


fig. 3.

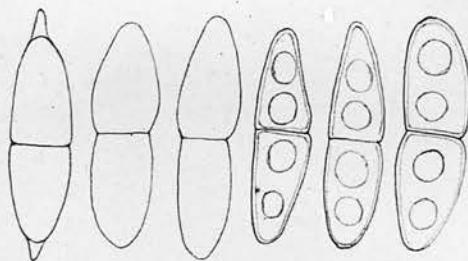


fig. 4.

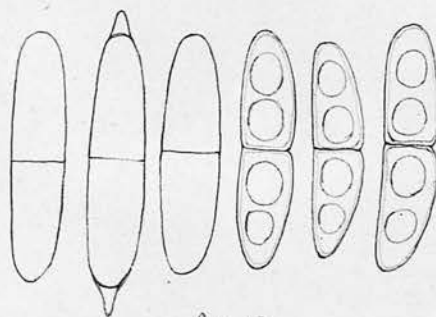


fig. 5.

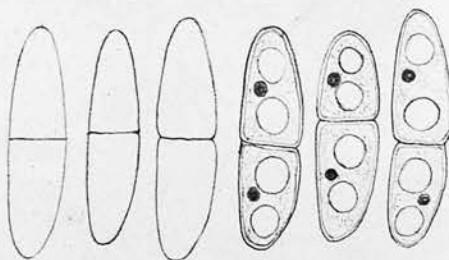


fig. 6.

Plate IV



fig 1.

Plate V



fig 1.

Plate VI

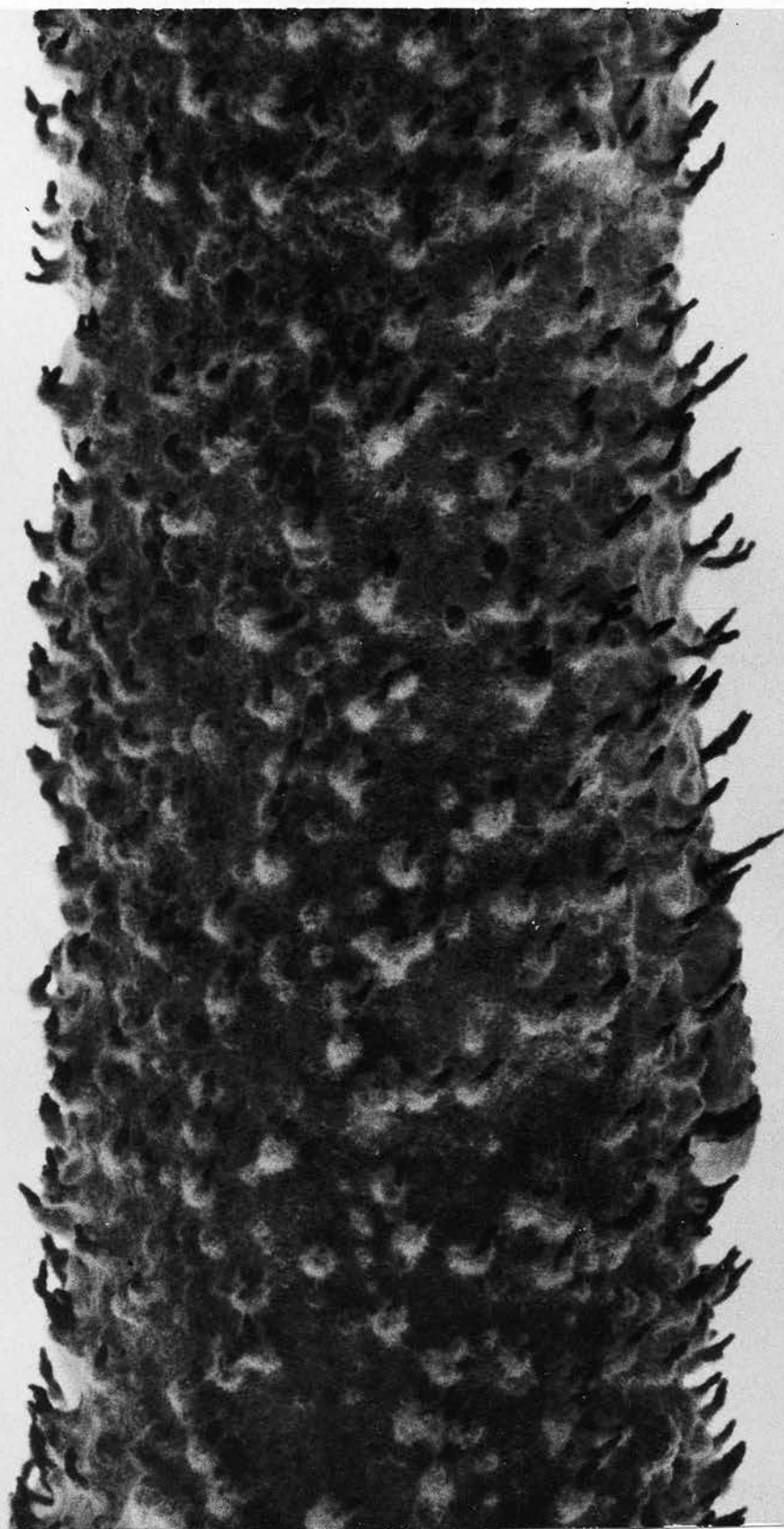


fig 1.

Plate VII

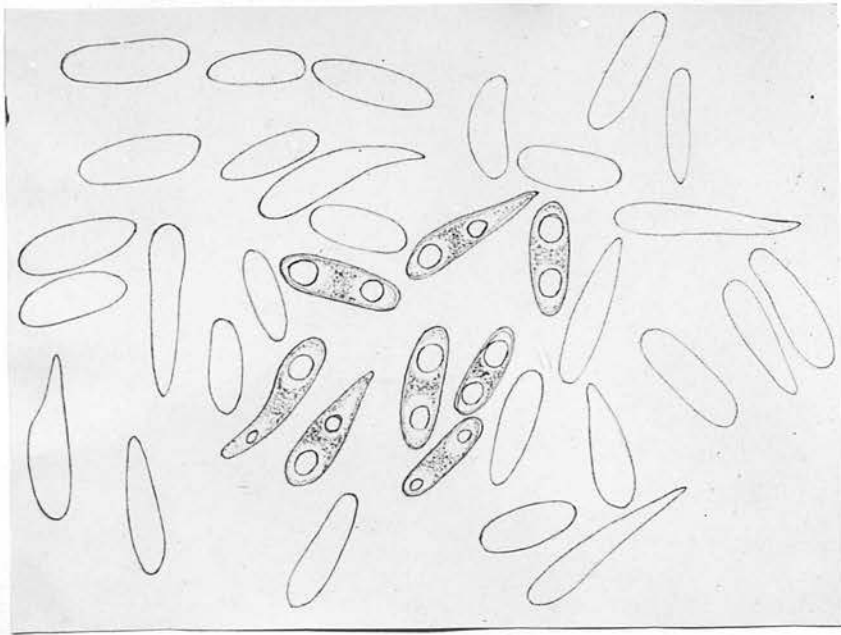


fig 1.

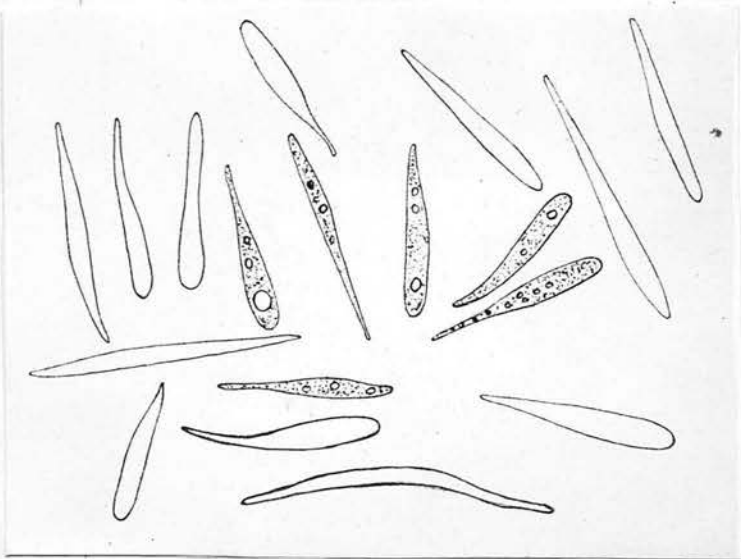


fig 2.

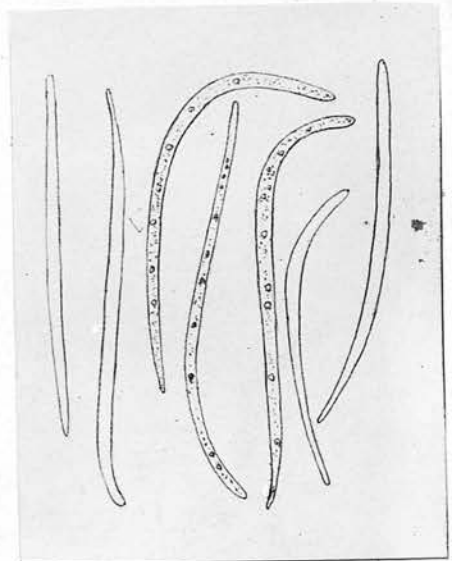


fig 3.

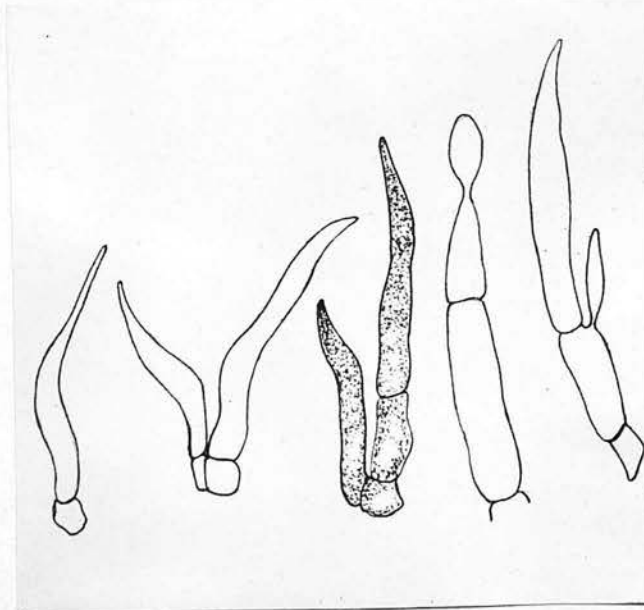


fig 4.

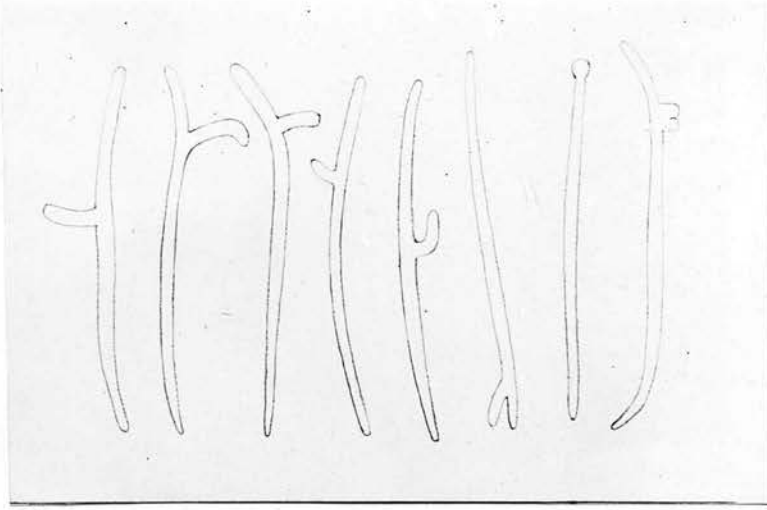


fig 1.

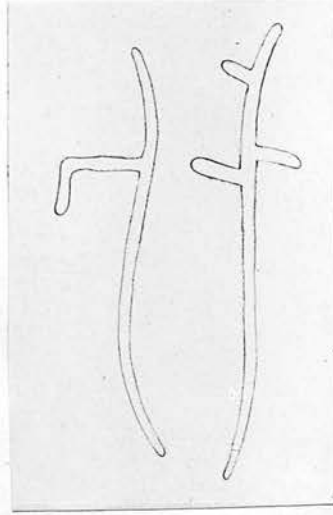


fig 2.

Plate IX

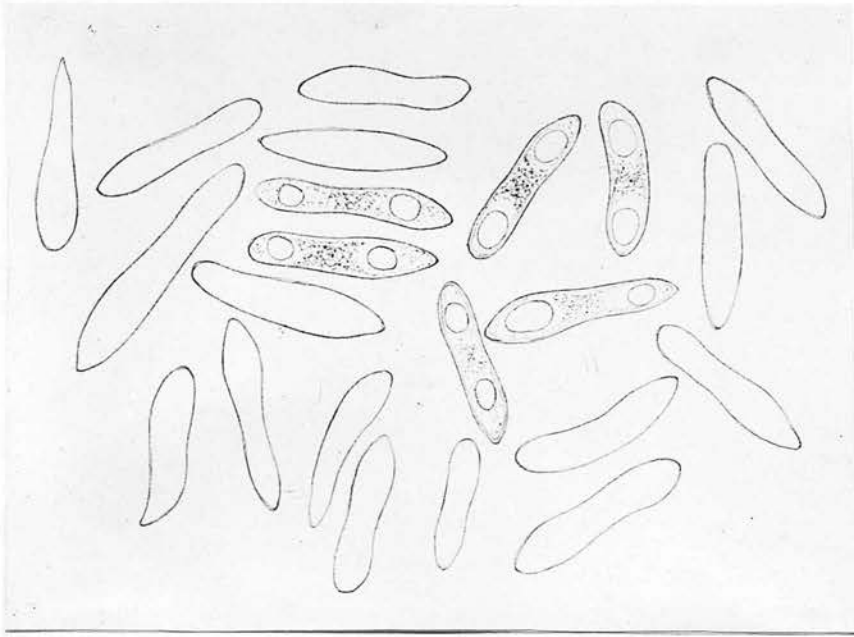


fig 1.

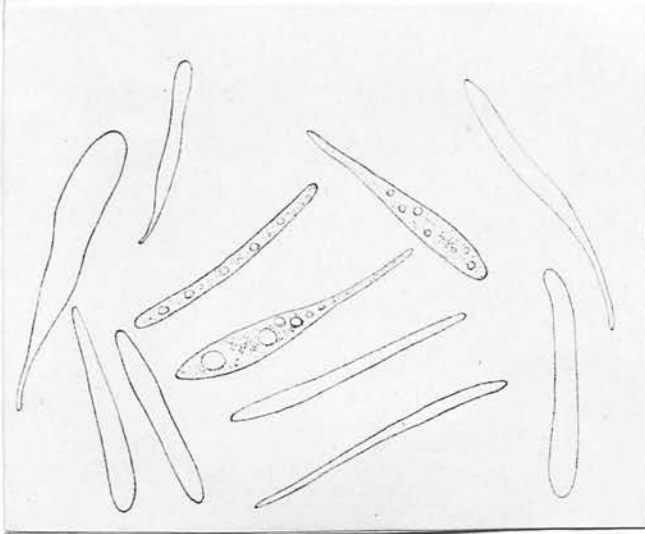


fig. 2.

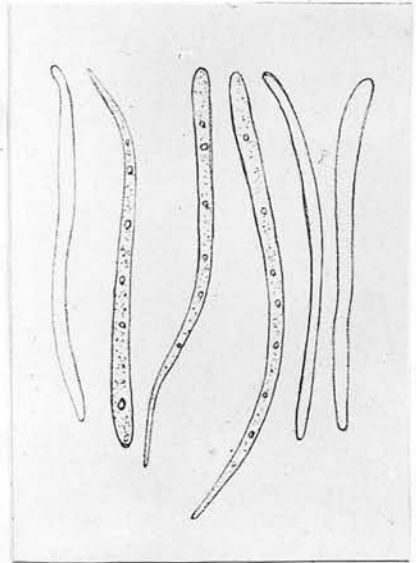


fig. 3.

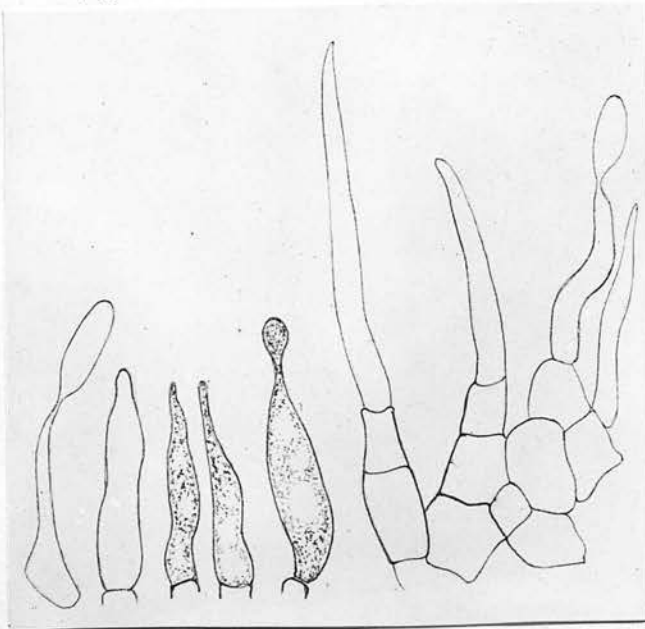


fig. 4.

Plate X

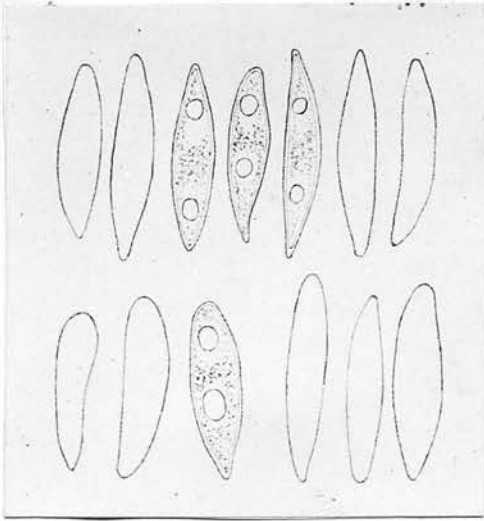


fig. 1.

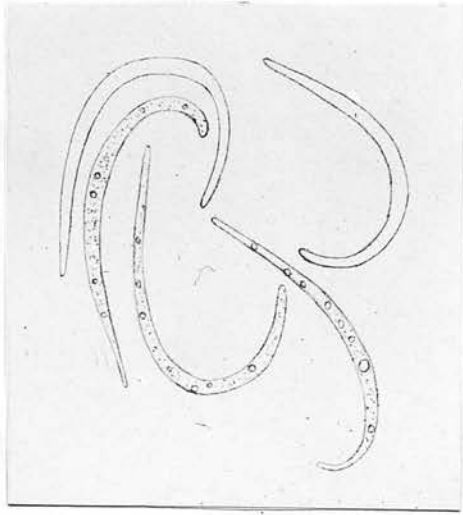


fig. 2.

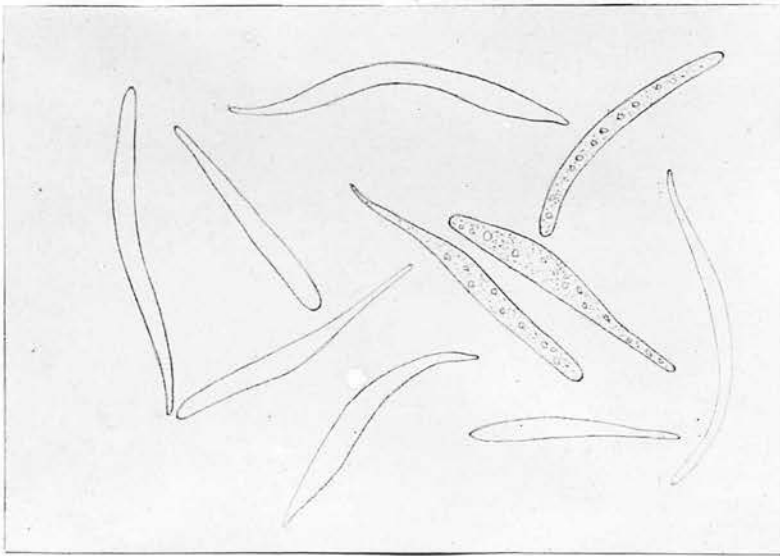


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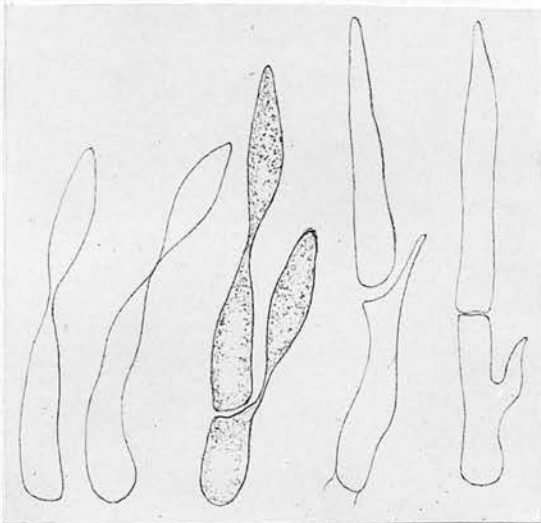


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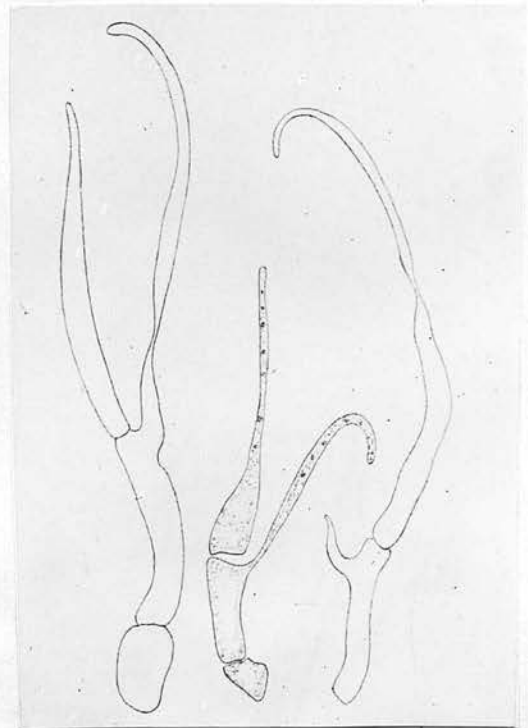


fig. 5.



fig 1.

Plate XII

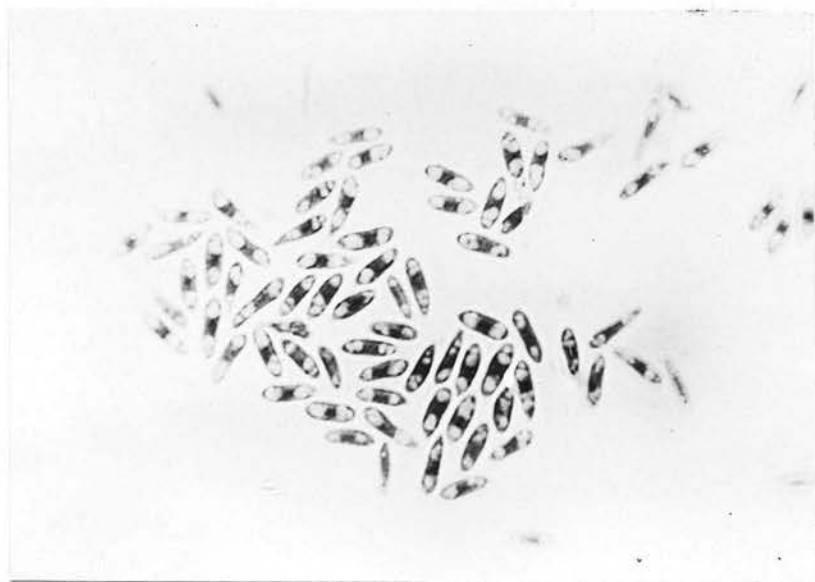


fig. 1.



fig. 2.

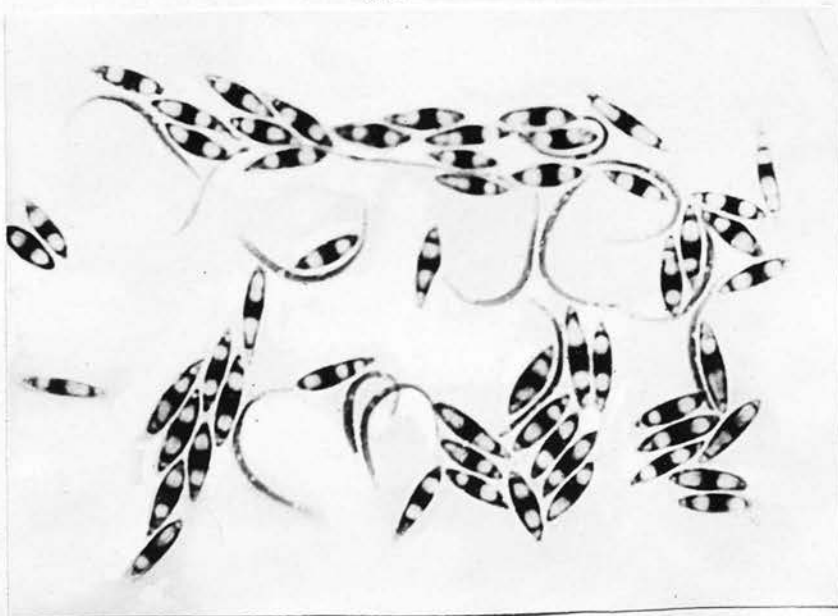


fig. 3.

Plate XIII

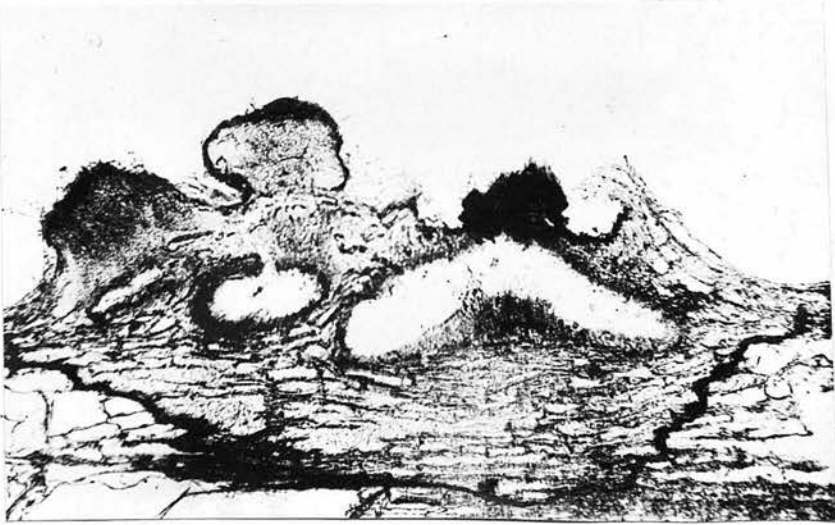


fig 1.

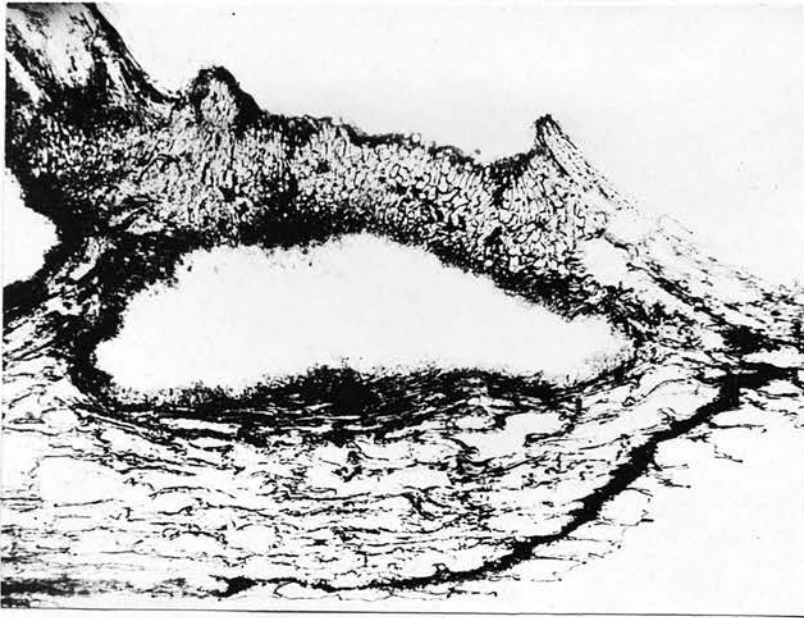


fig. 2.

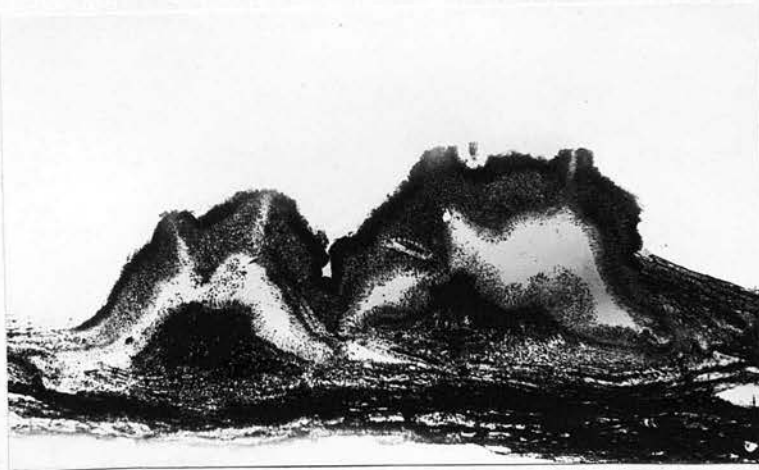


fig 3.

Plate XIV

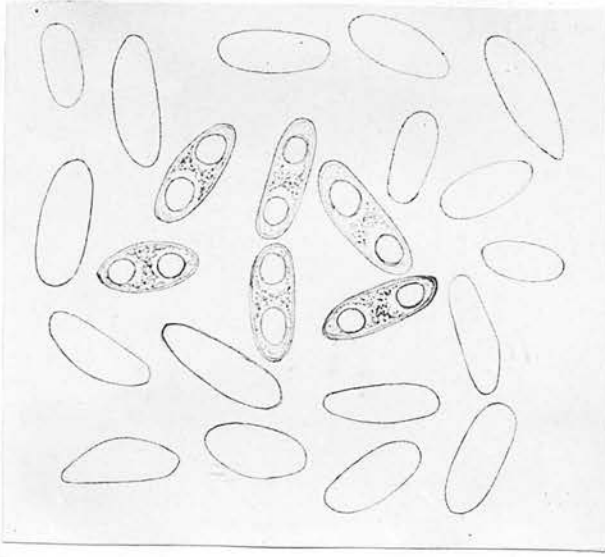


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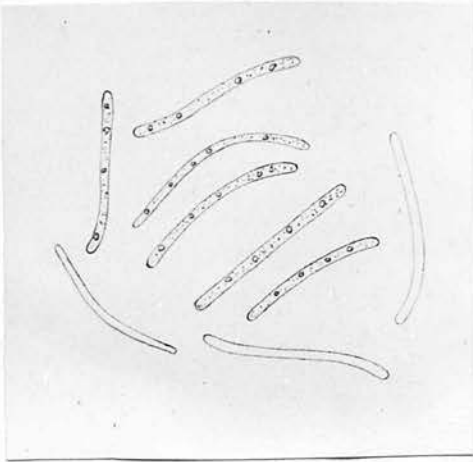


fig. 2.

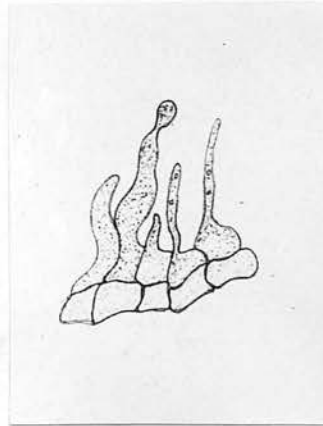


fig. 3.

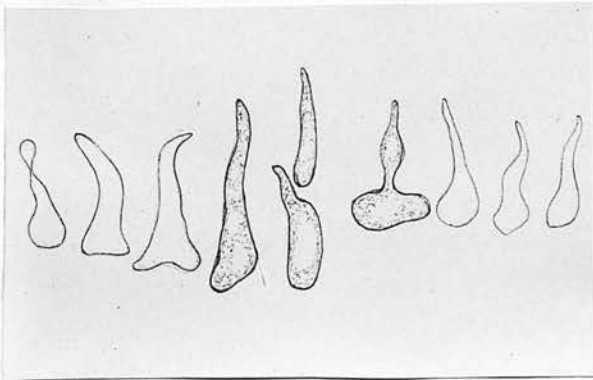


fig. 4.

Plate XV

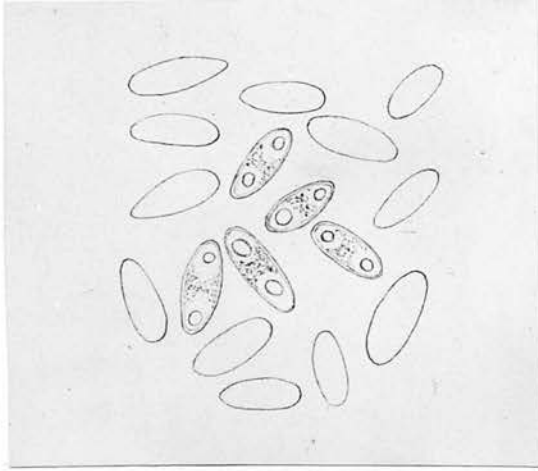


fig. 1.

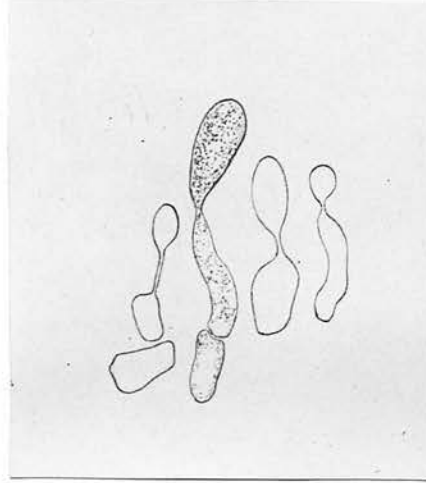


fig. 2.

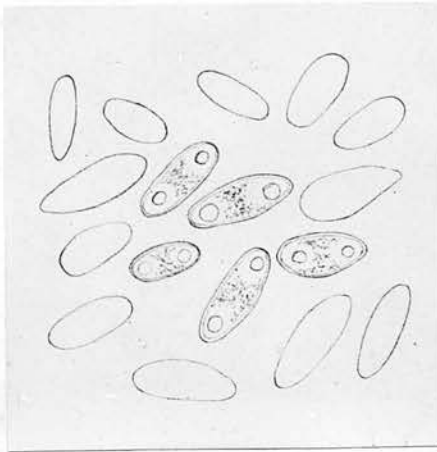


fig. 3.

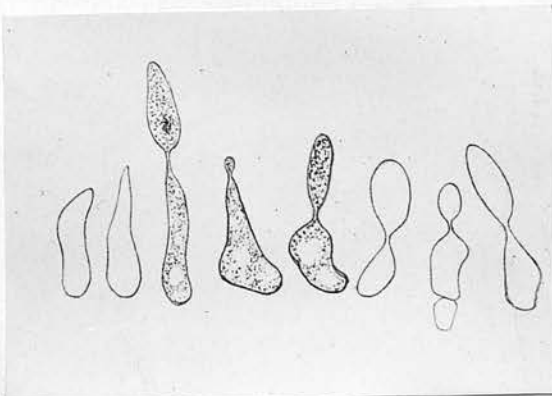


fig. 4.

Plate XVI

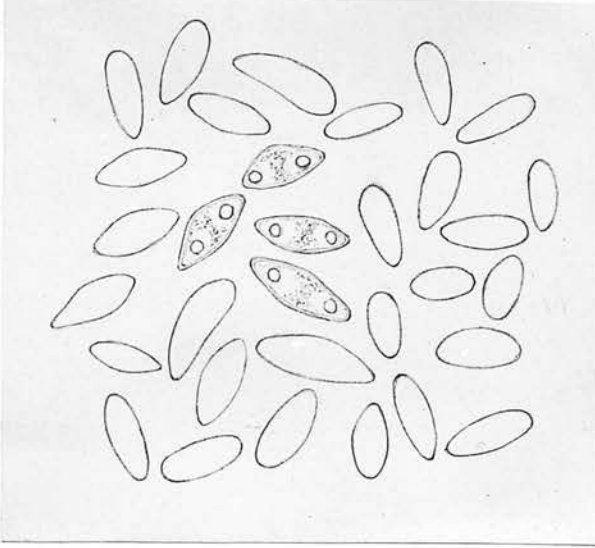


fig. 1.

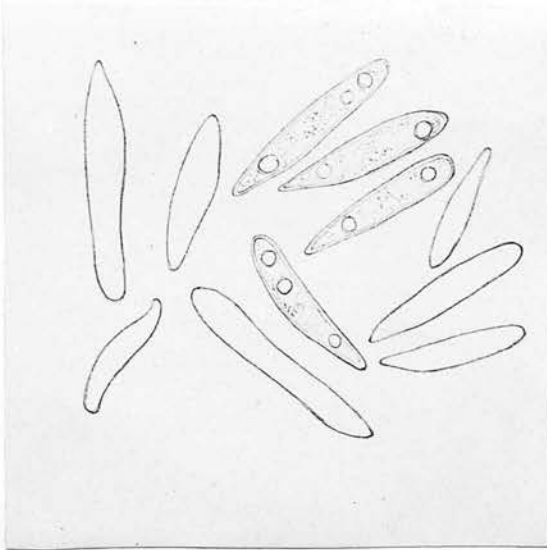


fig. 2.

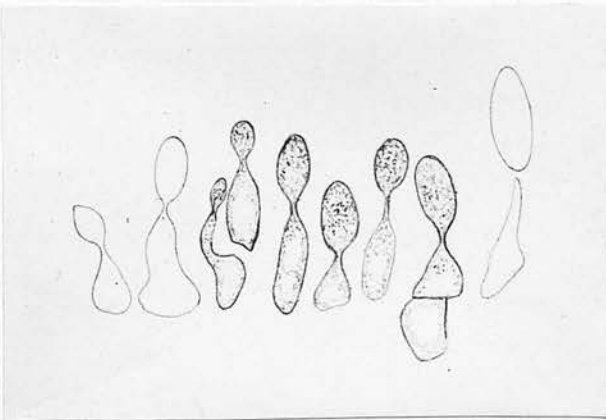


fig. 3.

Plate XVII

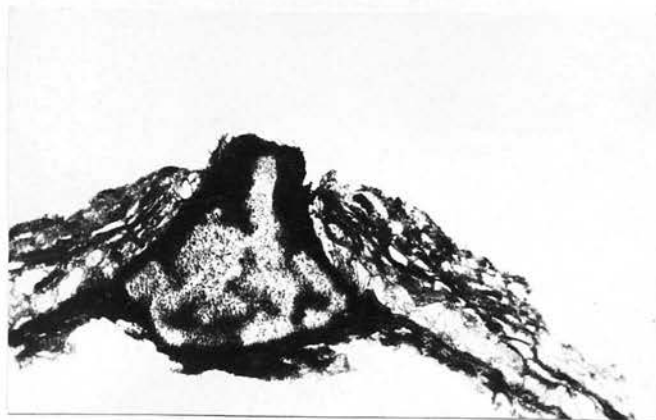


fig. 1.



fig. 2.

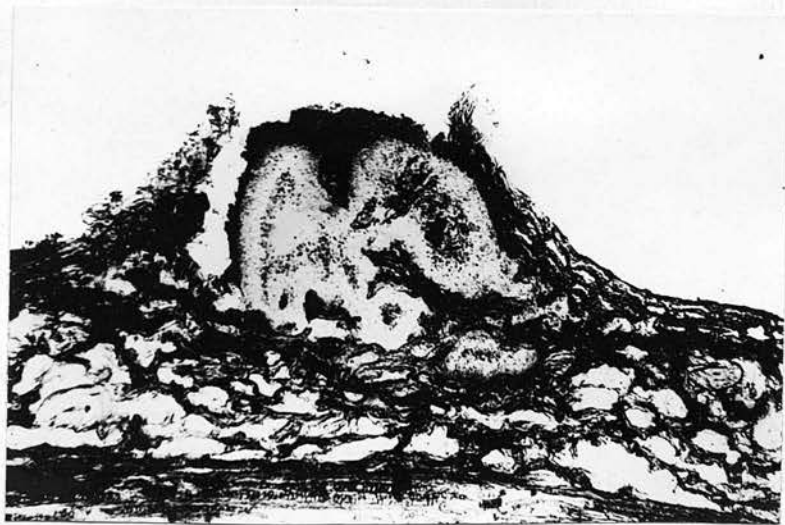


fig. 3.

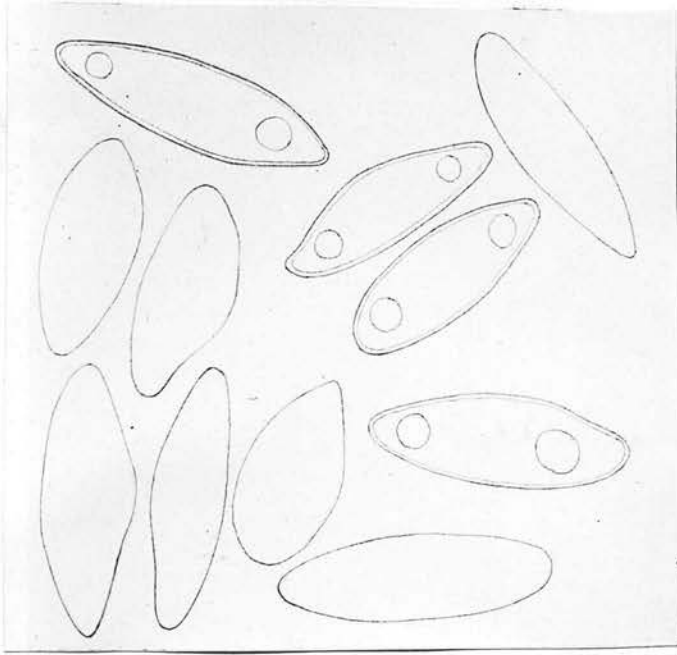


fig. 1.

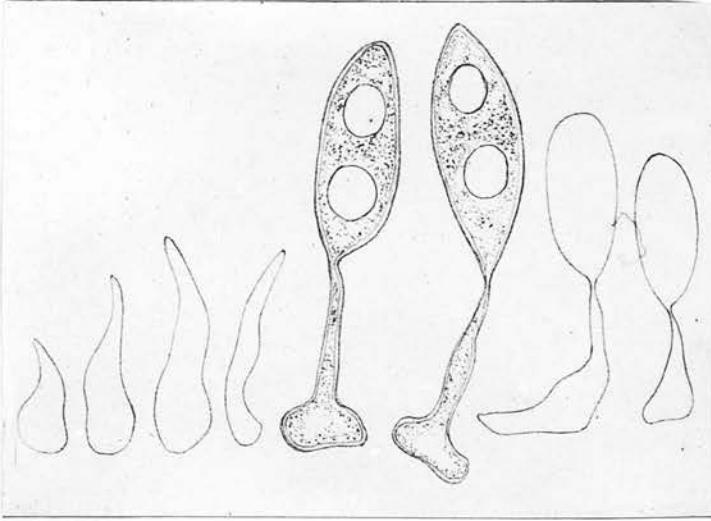


fig. 2.

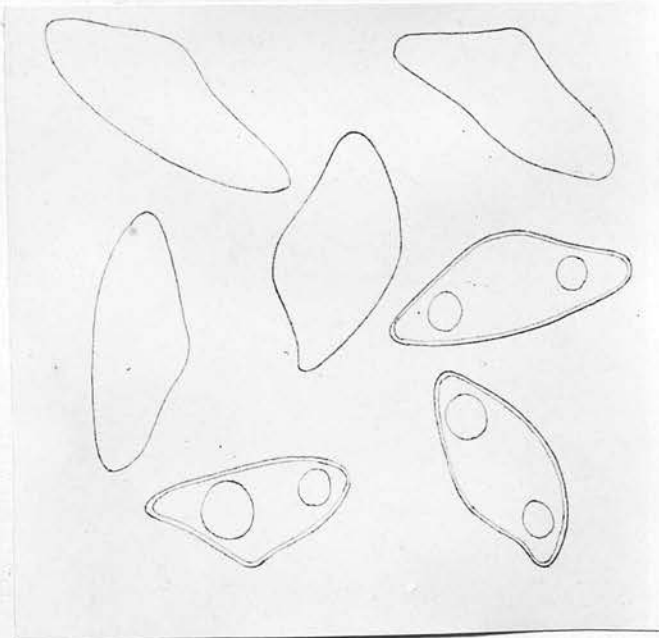


fig. 3.

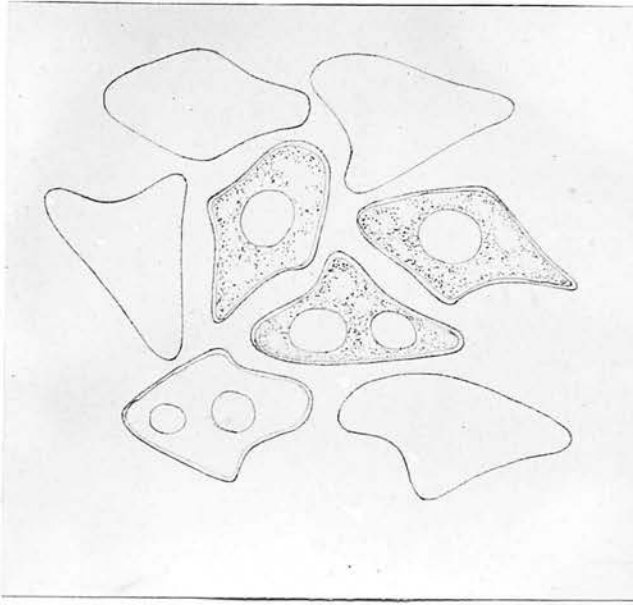


fig. 1.

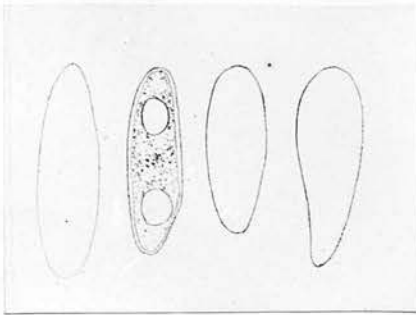


fig. 2.

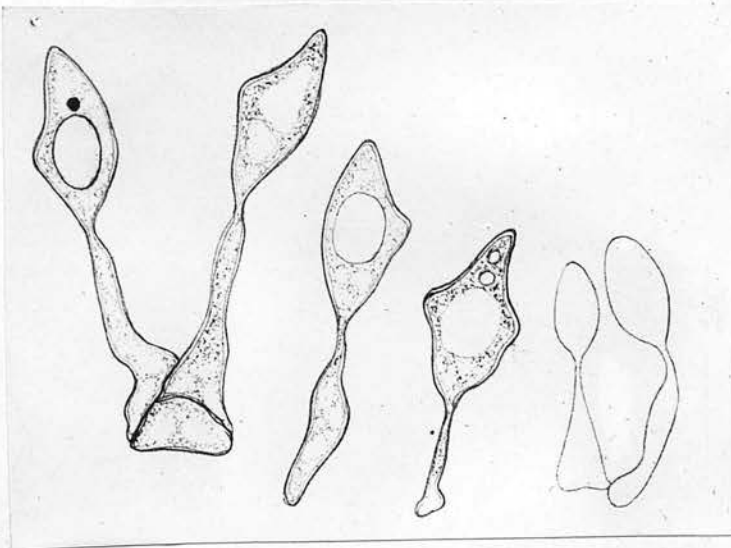


fig. 3.

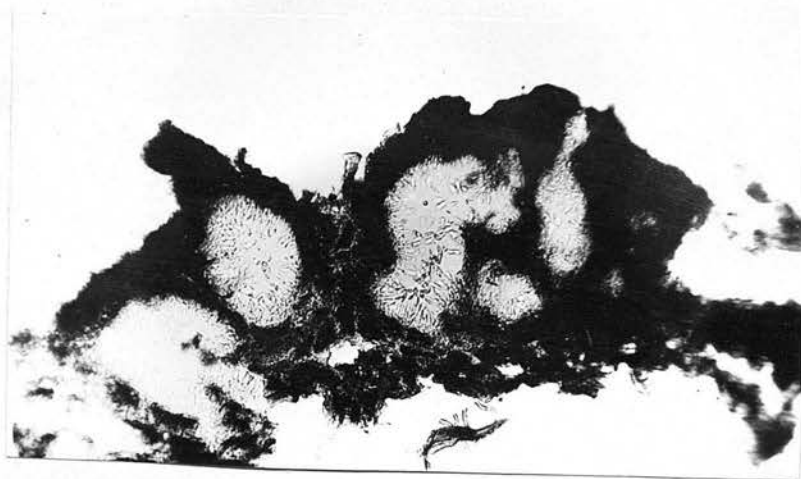


fig. 1.



fig. 2.

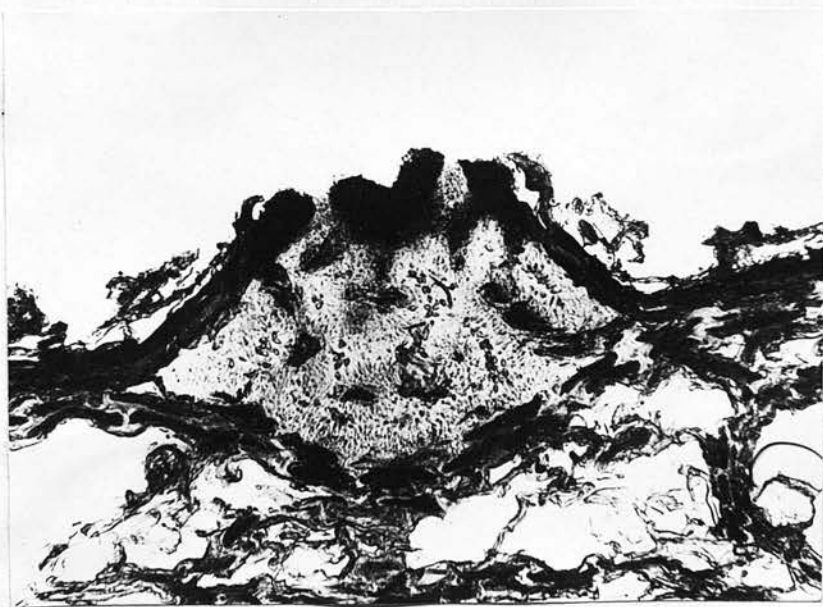
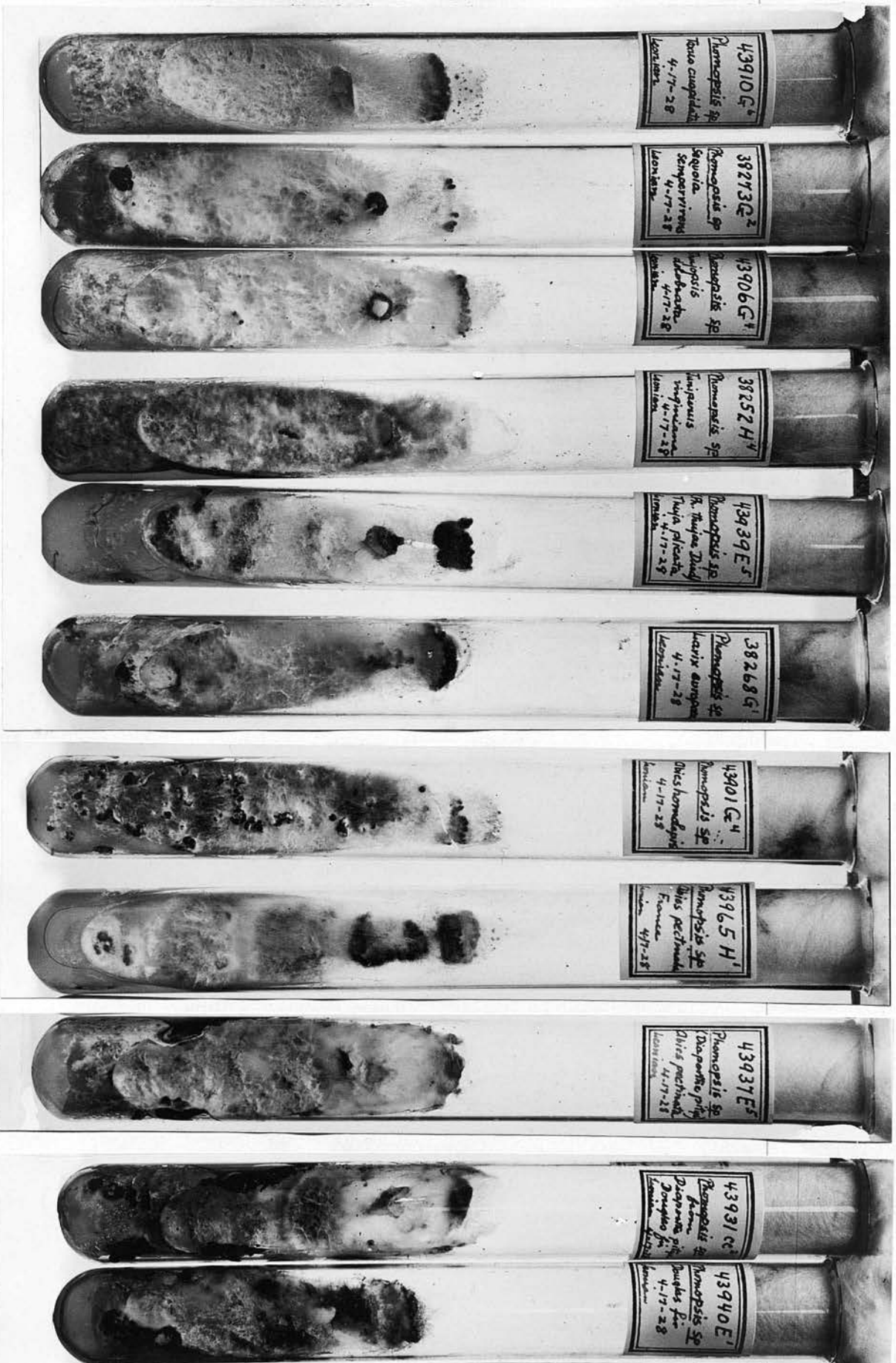
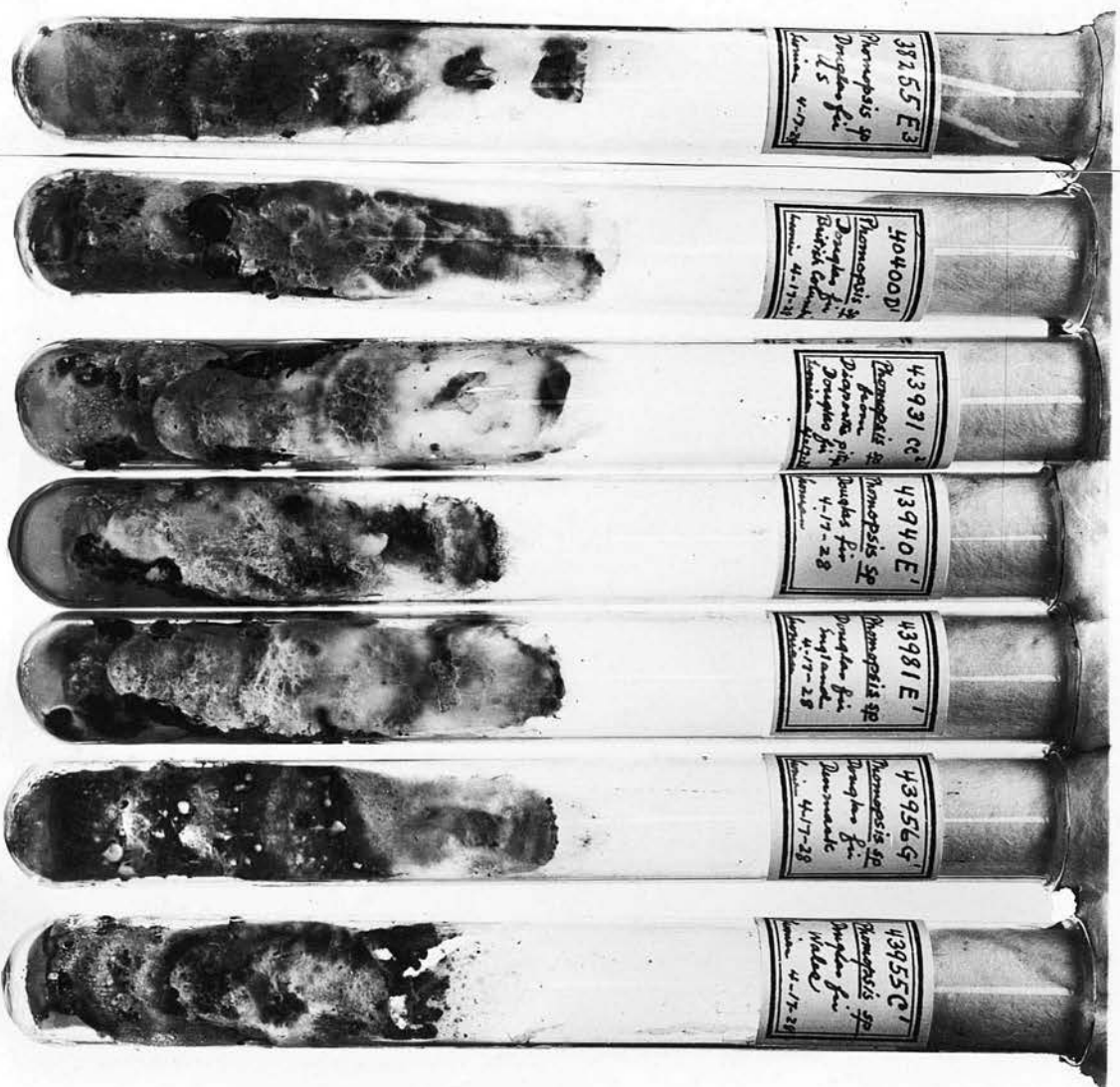


fig. 3.





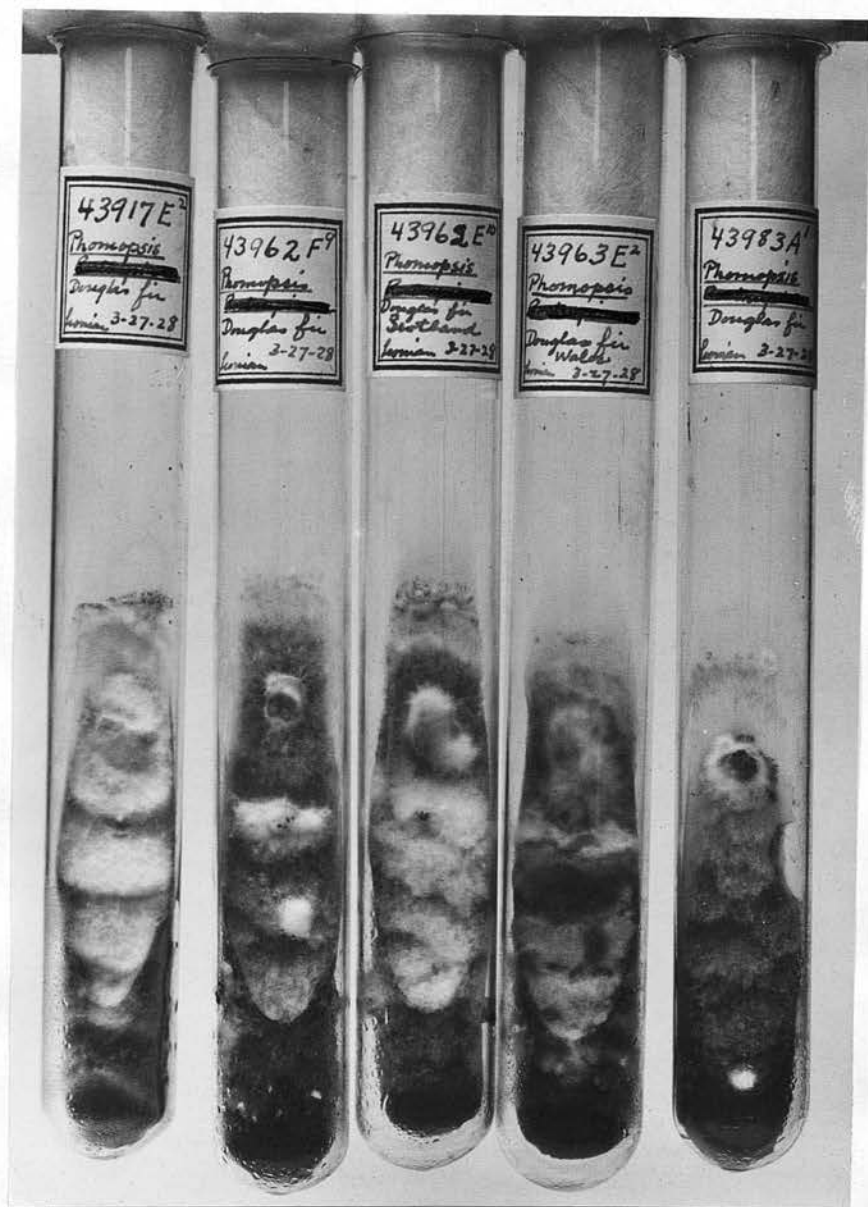




fig. 1.

fig. 2.

fig. 3.

fig. 4.